PONTIFICIA UNIVERSIDAD CATÓLICA DE CHILE
Facultad de Ciencias Biológicas
Programa de Doctorado en Ciencias Biológicas
Mención Ecología

TESIS DOCTORAL:

TAXONOMÍA, FILOGENIA Y FILOGEOGRAFÍA DE LA FAMILIA SCYTOSIPHONACEAE (PHAEOPHYCEAE), CON ÉNFASIS EN EL ORIGEN Y DISTRIBUCIÓN DEL GÉNERO *Scytosiphon* EN LA COSTA PACÍFICO SURESTE.

Por

CAROLINA CAMUS TORRES

Junio 2013

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Tesis presentada a la Pontificia Universidad Católica de Chile como parte de los requisitos para optar al grado de Doctor en Ciencias Biológicas mención Ecología.

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La defensa final de la tesis Doctoral titulada:

Taxonomía, filogenia y filogeografía de la familia Scytosiphonaceae (Phaeophyceae), con énfasis en el origen y distribución del género *Scytosiphon* en la costa Pacífico Sureste.

Presentada con fecha de hoy por el candidato a Doctor

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Ha sido aprobada por el Tribunal Examinador, constituido por los profesores abajo firmantes, calificándose el trabajo realizado, el manuscrito sometido y la defensa oral con nota _____

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A mi mono y monitas:

Javier, Ignacia, Francisca y Pili

AGRADECIMIENTOS

Agradezco a las entidades que hicieron posible el desarrollo de esta tesis doctoral a través de su financiamiento: 1) Comisión Nacional de Ciencia y Tecnología (CONICYT) por las Becas de Doctorado y de Termino de Tesis Doctoral; 2) A la Vicerrectoría Adjunta de Investigación y Doctorado (VRAID), de la Pontificia Universidad Católica de Chile, por la beca de Apoyo a la Participación en Congresos Internacionales, y 3) Departamento de Ecología & Center of Advances Studies in Ecology and Biodiversity (CASEB), proyecto FONDAP 1501-0001 Programa 7.

Especialmente quiero agradecer a mis tutores Dr. Sylvain Faugeron y Dr. Juan Correa por su constante dedicación en mi formación, por el apoyo que siempre me entregaron y por la tremenda confianza depositada en mí y en mi trabajo. Muchas gracias por todo!

Agradezco a mis compañeras del Laboratorio de Algas y de Diversidad Molecular, por su espíritu crítico y constructivo en las discusiones y constante ayuda en el aprendizaje de nuevas técnicas. Muy especialmente a mis compañeras y amigas Jessica Beltrán, Martha Hengst y Alejandra González, por su apoyo más allá del trabajo de esta tesis. Nunca olvidare los cafes en las mesas de abajo!

A mis padres, por darme aliento, transmitirme su fortaleza y enseñarme que los objetivos que uno se propone se consiguen con esfuerzo y son los más valorados. Gracias mamá y papá!

A mi hermana Eu y a Jan por su constante apoyo y preocupación. GRACIAS!!!!!

Finalmente, quiero agradecer a mi familia. A mi marido Javier, por aceptar mi dedicación a la tesis, mis eternos monólogos sobre *Scytosiphon*, mis pataletas frente al computador y por recibirme con un beso y almuerzo/comida cada día. Muchas gracias Momi, SIN TI esto no

hubiera sido posible!!!!! A mi hija Ignacia por aguantar mi ausencia en sus primeros meses y por supuesto a mi Pili por que siempre estuvo conmigo!

CONTENTS

ABBREVIATION LIST

AIC: Akaike information criterion
ANOSIM: Analysis of similarities
ANOVA: Analysis of variance
BI: Bayesian inference
BSA: Bovine serum albumin
COX3: Cytocrome oxidase 3
DNA: Deoxyribonucleic acid
GTR+I+G: General time reversible model
G: Shape parameter of the gamma distribution
HKY+I+G: Hasegawa, Kishino and Yano model
I: Proportion of invariable sites
ITS1: Internal transcribed spacer 1
LRT: Likelihood ratio test
ML: Maximum likelihood
MP: Maximum parsimony
mtDNA: mitochondrial deoxyribonucleic acid
MCMCMC: Metropolis-coupled markov chain monte carlo
nMDS: non-metric multidimensional scaling
PHT: Partition homogeneity test
*rbc*L-spacer-*rbc*S: large subunit-spacer-small subunit of the Rubisco gene
SIMPER: Similarity percentage
SSCP: Single-strand conformation polymorphism
TAE: Tris acetic acid-EDTA
TBE: Tris boric acid-EDTA
TBR: Tree bisection reconnection
TIM+I+G: Transitional model

GENERAL INTRODUCTION

Reactions to the ongoing biodiversity crisis are ubiquitously phrased in terms of species. Areas of importance (e.g. biodiversity hotspots) are selected on the basis of the number of species they possess. Conservation schemes are assessed on how many species are preserved. Lists are compiled of endangered species and the factors that threaten them. And conservation legislation and politics are focused on species preservation (Agapow et al., 2004). Species are commonly perceived as units of biodiversity participating in natural processes and the focus of many research paradigms. Their delineation and characterization are fundamental to the natural sciences (Mayden, 2002). Despite that biodiversity is measured in terms of species, the very term "species" is deeply ambiguous. While biologists customarily treat species as tangible entities, there is nonetheless a vast spectrum of meanings attached to the word. The argument over how species should be defined is endless, with over twenty species concepts under use at present (de Queiroz, 2005; 2007; Hey, 2001). These concepts encompass many operational and empirical definitions, often resulting in a given group of organisms being viewed in drastically different ways by different workers (Agapow et al., 2004). The potential conflicts posed by the use of different definitions for the term species are not just questions of semantics or miscommunication because species are routinely used as fundamental units of analysis in biogeography, ecology, macroevolution and conservation biology, and a better understanding of these larger scale processes requires that systematic employ methods to delimit objectively and rigorously what species are (Sites and Marshall, 2003).

Different methods have been proposed to solve the problem of delimiting species (e.g. cladistic haplotype aggregation, genetic distance and

phylogenetic/composite tree-based method; for review see Sites and Marshall, 2004), instead of identifying the species concept that best suits the specific questions that each researcher is trying to solve. When we used different methods, another impediment arises: different methodologies arrive at different boundaries. For example, it is very likely that the number of species that will be recognized by employing the phylogenetic species concept will be different relative to the number of taxa that have been recognized using the morphological species concept. Finally, it is necessary to note that the species concept that we choose implies that we determined a limit of species that is not universal, but particular to our problematic.

The "problem of species" involves all life forms, but their impact is particularly strong within complex groups such as seaweeds. The phycological literature is repleted of cases were species delimitation or the methodological problem of identifying the boundaries between a set of species is the issue (e.g. *Sargassum* species (Cheang et al., 2008), the genus *Enteromorpha* (Blomster et al., 1998), the kelp *Alaria* (Lane et al., 2007), the genus *Dictyota* (Tronholm et al 2010), the *Scytosiphon* complex). Traditionally the classification at the species-level is centered on the morphological species concept, based explicitly or implicitly on the detection of morphological discontinuities in sets of field-collected or cultured organisms (Wattier and Maggs, 2001), restricted to the definition of a type specimen in a type locality that serves as central reference for comparisons (Tautz et al., 2003). However, the biological species concept is difficult to apply in macroalgae partly because incomplete reproductive isolation is probably the rule for many seaweeds since hybridization has been demonstrated between species of a genus (e.g. *Macrocystis* (Lewis and Neushul, 1994) and *Fucus* (Coyer et al., 2002)) and between species of different genera (*Macrocystis* with *Pelagophycus* (Lewis and Neushul, 1995) and *Alaria* with *Lessoniopsis* (Liptack

and Druehl, 2000)), or because populations of some species have been found to suffer from being asexual (*Fucus vesiculosus* (Tatarenkov et al., 2005) and *Scytosiphon lomentaria* (Kogame et al. 2005)). In addition, traditional algal taxonomy most often ignores the ecological and genetic variability within the distribution of the species.

The 80`s witnessed a change from largely typological and phenetic approaches to cladistic methods of classification based on homology and the strict recognition of monophyletic groups (Medlin et al., 2007). Hence, the phylogenetic species concept, which defines a species as a monophyletic group composed of "the smallest diagnosable cluster of individual organisms within which there is a parental pattern of ancestry and descent", was introduced in algae (Medlin et al., 2007). This concept offers the advantage of being applied to asexual species and the opportunity to look at relationships over time by studying different depths in a phylogenetic tree, as opposed to the biological species concept which can only be used with sexually reproducing living organisms. The problem is that, in practice, the morphological species concept is still used as basis for species-level and intraspecific studies in macroalgae, while molecular data assist in calibrating or testing the limits and defining boundaries between morphologically defined species (Wattier and Maggs, 2001). This is a bad practice because, for example, brown algae in general reveal small-scale patterns of genetic differentiation according to what is expected from their poorly dispersing propagules. *Fucus vesiculosus* showed genetic differentiation at 10 m of distance (Tatarenkov et al., 2007) and *Postelsia palmaeformis* exhibits genetic differences at 1-6 m (Coyer et al. 1997). This means that due to the high intraspecific genetic structure, it is not the same to consider any individual as reference to define the boundaries of the species with which we are working. Furthermore, phenotypic plasticity is quite common in macroalgae, leading to changes in morphology and life cycle, which eventually leads to

consider them as different species (*Macrocystis pyrifera*, Demes et al 2009). In 2005, Bergstrom et al. observed a dwarf *F. vesiculosus* living in sympatry in the Baltic sea with the common morphotype. After genetic and morphological studies, they reported that the dwarf morph corresponds to a new species: *F. radicans* who, unlike *F. vesiculosus*, reproduces mostly asexually.

In summary, currently the traditional approach for species identification has four significant limitations as mentioned by Herbert et al. (2003). First, both phenotypic plasticity and genetic variability in the characters employed for species recognition can lead to incorrect identifications. Second, this approach overlooks morphologically cryptic taxa, which are common in many algal groups (Knowlton, 1993). Third, since morphological keys are often effective only for a particular life stage or genus, many individuals cannot be identified. Finally, the use of keys often demands such a high level of expertise that misdiagnoses are common. The inherent limitations of morphology-based identification systems and the dwindling pool of taxonomists signal the need for a new approach to taxon recognition: sequence-based species delimitation or barcodes (Herbert et al., 2003). These later approximations based on molecular markers are indeed a real contribution, but instead of considering this approach as "the solution" to morphology-based delimitation, it would be wiser to consider these approaches not as a separate tool, but as a part of an integrated approach.

This change of view is fundamental because in recent years, molecular tools have contributed significantly to the understanding of both historical and contemporary processes that affect lineages of macroalgae. For example, the Last Maximum Glacial (20 000 – 18 000 years ago) dramatically affected extant distributions of several macroalgae. In the southern hemisphere, Fraser et al. (2009) reported, based on mitochondrial COI and plastidial *rbc*L, that the bull kelp *Durvillaea antartica* was

eliminated from it subantartic distribution and only recently recolonized this region. While, in the northern hemisphere, Hoarau et al. (2007), also based on mitochondrial DNA, recognized three glacial refugia for *Fucus serratus*, and identified that the Irish refugium was the source for a recolonization of its current range. Also, population genetics analyses contribute to the detection and understanding of recent events and biological invasions. For example, using microsatellite, Billot et al. (2003) explored the roles of dispersal and habitat discontinuities in shaping the genetic structure of *Laminaria digitata*. They reported that the populations of the kelp were geographically structured following a pattern of isolation by distance and habitat discontinuities accentuate this differentiation. Also, through population genetic analyses of *Undaria pinnatifida*, Voisin et al. (2005) and Uwai et al. (2006), where able to study genetic diversity of native and introduced population worldwide and recognized different processes and route of introductions. Establishing the event(s) that shaped the distribution of a certain species is necessary to include it within its delimitation because these are features that contributed to define what a species is. Considering the phylogeographic context of a species within its delimitation changes our conception of that species because now we also are able to define it in its historical and contemporary context.

In this context, the phaeophycean family Scytosiphonaceae is an outstanding model to delve in the species problem and the origin of species distribution. It is a group with a notoriously long history of confusing species circumscription mainly because of a lack of reliable taxonomic diagnostic characters due to the simple architecture of the thallus, the use of different characters by different authors to refer to the same species and to the high morphological variability displayed by the thallus. At the molecular level, several authors have suggested that the family requires a revision due to the lack

of agreement between the phylogeny and the current taxonomic classification, principally because the monophyly of most genera comprising the family is questionable. This is a consequence of morphology-based species delimitation, because morphological characters used in identification need not *per se* be phylogenetically informative, i.e. synapomorphic. Phylogenetic classification needs to reflect the evolutionary history of identity by descent through homology (Medlin et al., 2007).

Another feature that makes this family an exemplary case is the contrasting distribution that the species that composed it present. Various species of the genera are widely distributed in temperate and cold waters throughout the world, while others present restricted distributions or even located in a specific locality of the world (e.g. *Scytosiphon crispus* in Falkland Island). Although the geographic distribution of species reflects the influence of historical and contemporary processes, as well as anthropogenic mediated introductions, the processes that have shaped the current distribution of these species have not been studied at all.

This thesis was divided in two chapters. The objective of the first one was to assess the current taxonomic status of the species belonging to the most confusing genera of the family Scytosiphonaceae along the Southeastern Pacific coast through a morphological and molecular approach and, as a second goal, to determine the validity of the diagnostic morphological characters currently in use. Due to the fact that in the first chapter it was clear that the genus *Scytosiphon* was one of the most confusing, the objective of the second chapter was to assess the genetic diversity of the *Scytosiphon* complex, based on a mitochondrial marker, to determine the level and pattern of genetic variation within species and between the species within the genus, to infer the historical-geographic origin of the species complex.

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CHAPTER 1

A molecular and morphological assessment of Scytosiphonaceae (Phaeophyceae) in the Southeastern Pacific

ABSTRACT

Due to the extremely simple morphology of members of the Scytosiphonacea, defining generic and species boundaries is troublesome. Morphological characters used to define species within these genera, often influenced by environmental factors, vary within and among populations. On the other hand, phylogenetic studies have utilized nuclear and plastidial molecular markers to explore relationships of the Scytosiphonales at the order and family level. However these phylogenetic studies have failed to solve the relationships within the group. The aim of this study was to assess the taxonomic status of the species belonging to the most confusing genera of the family found in the Southeastern Pacific coast through morphological and molecular approaches. As a second goal, I evaluated if correspondence does exist between morphological and molecular species to determine the validity of the morphological characters used for species diagnoses. Our molecular results, based on nuclear, plastidial and mitochondrial markers, demonstrated that the genera *Endarachne*, *Petalonia* and *Scytosiphon* are paraphyletic. Moreover, we detected a *Scytosiphon* complex which harbours a large cryptic diversity along the Chilean coast, including three distinct lineages of *S. lomentaria* and a possible new species endemic to the region. Morphological analyses revealed strong similarity between species of *Scytosiphon* and between species of *Petalonia* and *Endarachne* which resulted in extreme overlapping of external and internal characters, indicating that they are not diagnostic. Considering both morphological and molecular features and to reflect more clearly the relationships between species, I propose to reclassify *E. binghamiae* and *P. fascia* within the genus *Scytosiphon*.

RESUMEN

Debido a la extrema simpleza de la morfología de los miembros de la familia Scytosiphonaceae, definir los límites de las especies y géneros es complejo. Los caracteres morfológicos utilizados para definir especies dentro de estos géneros, usualmente influenciados por factores ambientales, varían entre y dentro de las poblaciones. Los estudios filogenéticos han utilizado marcadores moleculares nucleares y plastidiales para explorar las relaciones entre Scytosiphonales a nivel de orden y familia. Sin embargo estos estudios filogenéticos no han podido resolver las relaciones dentro de este grupo. El objetivo de este estudio fue evaluar el status taxonómico de las especies que pertenecen a los géneros mas confusos de la familia que se encuentran en la costa del Pacifico Sureste a través de una aproximación morfológica y molecular. Como segundo objetivo, evalué la correspondencia entre especies morfológicas y moleculares para determinar la validez de los caracteres morfológicos usados para el diagnostico de las especies. Nuestros resultados moleculares, basados en marcadores nucleares, plastidiales y mitocondriales, demostraron que los géneros *Endarachne*, *Petalonia* y *Scytosiphon* son parafiléticos. Más aun, detectamos al complejo *Scytosiphon* el cual abarca gran diversidad criptica a lo largo de la costa Chilena, incluyendo tres distintos linajes de *S. lomentaria* y una posible nueva especie endémica para la región. Análisis morfológicos revelaron fuerte similitud entre las especies de *Scytosiphon* y entre las especies de *Petalonia* y *Endarachne*, las cuales muestran extrema sobreposición de caracteres externos e internos, indicando que estos últimos no son diagnósticos. Finalmente, considerando tanto los rasgos morfológicos y moleculares y para reflejar más claramente las relaciones entre especies, propongo la reclasificación de *E. bingamiae* y *P. fascia* dentro del género *Scytosiphon*.

1. INTRODUCTION

The classification system of the algal class Phaeophyceae at the ordinal rank has been revised several times. The ordinal delineation has been based on the type of life cycle, mode of growth, type of gamy and the type of thallus construction (i.e. filamentous *vs* parenchymatous) (de Reviers et al., 2007). Based on those characteristics, various systems of classifying the Phaeophyceae have been proposed (see Table 5 in de Reviers and Rousseau, 1999). Regardless which system is considered, one of the main disputes concerning brown algal classification has been whether to accept a narrower (*sensu stricto*) or a wider (*sensu lato*) circumscription for the Ectocarpales (Rousseau and de Reviers, 1999).

Our understanding of the classification of Ectocarpales has undergone marked changes since 1990 because of the contribution of molecular phylogenies. In 1999, Rousseau and Reviers made a formal new circumscription of the Ectocarpales *sensu lato*. According to this classification, the order corresponds to a monophyletic group that includes representatives of the formerly accepted order Ectocarpales *sensu stricto*, Chordariales, Dictyosiphonales and Scytosiphonales, all of which have one or several plastids with one or several stalked pyrenoids. The same authors considered stalked pyrenoids to be a synapomorphy (i.e. shared derived character) of the Ectocarpales and a diagnostic character of the order. Due to the reorganization within the order, and to reconcile nomenclature and phylogeny, Peters and Ramírez (2001) proposed a new concept for families in the Ectocarpales. This proposal reorganized the number of families within the order, where some of the originally accepted families maintained their status and others were merged. This was the case for the family Scytosiphonaceae. Classification of the Scytosiphonaceae has been based on the external morphology and structure of erect

thalli (Wynne, 1969; Kogame et al., 1999). Currently, the family includes 24 genera, six of which contain no accepted species and four are monotypic (Guiry and Guiry, 2012). These genera are distinguished mainly on the basis of external characteristics of the erect thallus (hollow versus solid, tubular, laminar or globular, branched versus nonbranched, with or without perforations) (Abbott and Hollenberg, 1976; Parsons, 1982). Species are mainly distinguished by the form and size of the erect thallus, number of cellular layers of the cortex and medulla, type of plurilocular sporangia, presence or absence of ascocysts and presence or absence of phaeophyceaen hairs (Wynne, 1969; Clayton, 1975; 1976; 1978; Abbott and Hollenberg, 1976; Wynne and Loiseaux, 1976; Pedersen, 1980; Parson, 1982; Kogame, 1998). However, ambiguities do exist for genera demarcation, particularly *Endarachne*, *Petalonia* and *Scytosiphon*, and also to distinguish species within those genera. Among the main reasons for these ambiguities is the lack of reliable taxonomic diagnostic characters due to the simple architecture of the erect thallus (Peréz–Cirera and Cremades, 1991; Wynne, 1969), the use of different characters by different authors to refer to the same species within each genus (Rhew and Boo, 1991; Lee et al., 1992; Pedersen, 1980), and to the high morphological variability displayed by the erect thalli (Clayton, 1976; 1978). A graphic example of this situation was provided by Guiry and Guiry (2012), who reported that, for the genus *Scytosiphon* only, 69 names of species where available in the literature, of which only 6 are currently accepted species and the others correspond to synonymies, species with uncertain taxonomic status and species with provisional names that have not been validated. Furthermore, even within the accepted species the limits are still not clear. All of these factors add to the current state of confusion in which the family is immersed.

Regarding the phylogenetic relationships within the family, few studies have reported phylogenies based on nuclear and plastid molecular markers (Kogame et al.,

1999; Cho et al., 2001; Cho et al., 2006). These studies have all suggested that the family requires a revision since the molecular results do not support the current taxonomic classification, because the monophyly of most genera comprising the family is questionable. However, Kogame et al. (1999) proposed that the morphological characters of the alternating phases (sporophytic) would better reflect the phylogenetic relationships. They argued that it is possible to recognize four groups based on the morphology of the sporophytes that coincide with the clustering observed on their phylogenetic constructs (*Compsonema*-like species, *Stragularia*-like species, species that produce plurilocular and unilocular sporangia, and species that produce only unilocular sporangia on its sporophytic thallus). More recently, Cho et al. (2006) concluded that Scytosiphonaceae consisted of two phyletic groups according to their reproductive organs (sporophytes with either unilocular sporangia only or with both plurilocular and unilocular sporangia), distribution patterns and molecular data. Based on these results, the authors proposed two taxonomic possibilities. One was to divide the family into two tribes (Scytosiphonieae and Chnoosporieae) and the other was to classify all members of the first phyletic group into the genus *Scytosiphon* and the second group into the genus *Hydroclathrus*, according to the priority of generic names. Thus, it seems clear that, until now, neither the phylogenetic nor the morphological approaches have been able to improve our knowledge of the family. Furthermore, delineation of the genera *Endarachne*, *Petalonia* and *Scytosiphon* remains ambiguous and current generic definitions do not reflect the phylogeny of the species.

In the Southeast Pacific coast only few species of the family have been reported (Romo and Alveal, 1977; Ramírez and Santelices, 1981; Santelices, 1989; 1991; Hoffman and Santelices, 1997). In 2005, Camus et al. added to the record the species *S. tenellus* and also showed that the traditionally described *S. lomentaria* distributed along the Peruvian-Chilean Pacific coast was not a single homogeneous entity as previously thought. Indeed,

Scytosiphon-like erect thalli from central Chile did not group with any of the previously reported *Scytosiphon* species, which led the authors to suggest that *Scytosiphon* from central Chile was probably a new species. Furthermore, erect thalli of *S. lomentaria* from Chañaral (northern Chile) were genetically close to those from Greece, suggesting multiple origins for what was considered so far as *S. lomentaria*. More recently, Contreras et al. (2007) reported the presence of *S. gracilis* with a highly likely origin in Korea. These reports suggest that traditional, morphology-based taxonomy of the family underestimated species diversity along the Southeastern Pacific coasts, and a careful re-examination is needed. Thus, the aim of this study was to re-assess the current taxonomic status of the species belonging to the most confusing genera of the family that are found in the Southeastern Pacific coast through a combination of morphological and molecular approaches. As a second goal was to evaluate if correspondence exists between morphological and molecular species' delineation, to determine the validity of the morphological characters used for species diagnoses.

2. MATERIALS AND METHODS

2.1. *Sampling*

Erect thalli of plants morphologically assignable to species of the genera *Colpomenia*, *Endarachne*, *Petalonia* and *Scytosiphon* were collected during low tide from intertidal rocky platforms in 33 localities along the Chilean coast (Table 1). In each locality, all the different morphologies found for each species were collected (Table 1). From each collected plant, a cleaned frond was immediately placed into a plastic bag with silica gel for rapid dehydration and the remaining tissue was preserved in a 10% formalin-seawater solution for morphological analyses. Vouchers numbers SSUC Nº7325 to SSUC Nº7522 were deposited in the Colección de Flora y Fauna Profesor Patricio Sánchez Reyes (SSUC) at the Departamento de Ecología, Pontificia Universidad Católica de Chile, Santiago, Chile.

2.2. *DNA sequencing*

Tissue processing started with small fragments of dried fronds placed in tubes with stainless steel beads, which were ground to fine powder in a Mini Beadbeater (Biospec Products, INC, Bartlesville, OK, USA). Total genomic DNA was extracted using a Ultra clean Soil DNA Kit (MoBio Laboratories, Carlsbad, CA, USA), following the manufacturer`s instructions. For almost all samples, three markers were analyzed: the nuclear marker internal transcribed spacer 1 (ITS1) was amplified using primers ITS1F and ITS1R (designed by Camus et al., 2005); the plastid marker partial *rbc*L-spacer partial *rbc*S was amplified using primers RS1and RS2 (designed by Lee et al., 1999), and the mitochondrial marker COX3 (cytocrome oxidase 3) was amplified using primers CAF4A and CAR4A (designed by Kogame et al., 2005). For all the markers, PCR reactions

(14.5μL total volume) contained 1μL of DNA, 10x reaction buffer (Invitrogen, Carlsbad, CA, USA), 50 mM $MgCl_2$ (Invitrogen), 2.5 mM of each dNTP (Fermentas, Burlington, Ontario, Canada), 5 U/μL Taq DNA polymerase (Fermentas), 10x BSA (New England, BioLabs, Ipswich, MA, USA) and 10 μM of each primer. The reaction profile for COX3 was 95°C for 8 min followed by 94°C for 1 min, 55°C for 45 sec, 72°C for 2 min and 35 cycles, and a final extension at 72°C for 7 min. For ITS1 the conditions were 95°C for 8 min followed by 94°C for 1 min, 57°C for 45 sec, 72°C for 2 min and 35 cycles, and a final extension at 72°C for 7 min. For partial *rbc*L-spacer partial *rbc*S conditions were 95°C for 8 min followed by 94°C for 1 min, 42°C for 30 sec, 72°C for 2 min and 25 cycles, and a final extension at 72°C for 7 min. PCR products were visualized with ethidium bromide staining after electrophoresis in a 2% agarose gel. If a single band was detected, the PCR product was purified with a QIAquick PCR purification kit (Qiagen, Duesseldorf, Germany) following the manufacturer's instructions or purified by Macrogen Korea. The sequences were determined using a Sequencer (3100 Genetic Analyser Applied Biosystems, CA, USA) or outsourced to Macrogen (Seoul, Korea). In total, 297 sequences were generated and submitted to GenBank (85 for ITS1, 95 for partial *rbc*L-spacer partial *rbc*S and 117 for COX3) (Table 1).

2.3. *Sequence alignments, data saturation and data congruency*

Chromatograms were inspected visually and the sequences were aligned using ClustalW (Thompson et al., 1994) implemented in BioEdit version 7.0.4.1. (Hall, 1999) or Multalin (Corpet, 1988). Accuracy of the phylogenetic signal was assessed using the *Iss* statistic according to Xia et al. (2003). The software DAMBE version 5.5.29 (Xia and Xie, 2001) was used to calculate *Iss* values and compare them against critical *Iss* values for

symmetric and asymmetric topologies (Xia et al., 2003). Since critical *Iss* values depend on the number of taxa and the sequence length and hence are dataset-specific and impractical to tabulate, DAMBE samples one thousand random subsets of 4, 8, 16, and 32 sequences from the alignment and calculates *Iss* for the subsets. When *Iss* statistic showed low phylogenetic signal, IndelCoder, implemented in SeqState version 1.4 (Müller, 2005) was used. With the growing number of studies that use length variable sequence regions, the inclusion of indel (insertion-deletion) characters in phylogenetic analyses is becoming more important (Müller, 2006) and it is widely recognized that indels are a valuable source of data for phylogenetic inference (Simmons et al., 2008). Only for ITS1 dataset, the *Iss* statistics was significantly higher than the critical values (*Iss.c*) for the alignment as a whole. To solve the substitution/saturation problem, indels were codified using IndelCoder. For COX3 and partial *rbc*L-spacer-*rbc*S datasets, the *Iss* statistics indicated no saturation (*Iss* < *Iss.c*), which allowed phylogenetic reconstructions to be based on the original dataset.

Partition homogeneity tests (PHT; Farris et al., 1995, Swofford, 2003) were run to determine the validity of using a single concatenated matrix for all DNA partitions. These tests were implemented in PAUP v.4.0b10 (Swofford, 2003) with 1000 replicates using a heuristic search with 10 replicates of random sequence addition and TBR branch swapping. A probability of 0.05 was considered as the threshold for significance. However, this test did not allow us to concatenate the three molecular markers because significant differences between the partitions were detected (P = 0,001). As a result, three separate dataset (ITS1, partial *rbc*L-spacer partial *rbc*S and COX3) were considered.

2.4. *Phylogenetic analyses*

Phylogenetic trees were constructed using both sequences of individuals collected along the Chilean coast (Table 1) and those available in GenBank for the rest of the world (Table 2). Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference (BI) methods were selected for the analyses. Akaike information criteria (AIC) and likelihood ratio tests (LRT) from Modeltest version 3.7 (Posada and Crandall, 1998) were used to determine the most appropriate model (For ITS1: HKY+I+G model (base frequencies A: 0.2074 / C: 0.2916 / G: 0.2713 / T: 0.2297; among site rate variation: 0.1697; gamma distribution shape parameter: 2.5105; Ti/tv ratio: 1.1716); for partial *rbc*L-spacer partial *rbc*S: HKY+G (base frequencies A: 0.3327 / C: 0.1486 / G: 0.1546 / T: 0.3641; gamma distribution shape parameter: 0.5312; Ti/tv ratio: 0.9879); and for COX3: TIM+I+G (base frequencies A: 0.2529 / C: 0.1671 / G: 0.1849 / T: 0.3952; substitution rates A-C: 1.0000 / A-G: 3.1920 / A-T: 1.2947 / C-G: 1.2947 / C-T: 4.9934 / G-T: 1.000; among site rate variation: 0.3549; gamma distribution shape parameter: 0.7942). ML and MP analyses were performed in PAUP*4.0b (Swofford 2003) and MEGA5 (Tamura et al 2011), respectively, for each dataset. ML was done using a heuristic search with random stepwise addition, tree bisection-reconnection branch swapping algorithm. Subsequently 1000 bootstrap replicates were analyzed. The MP tree was obtained using the Close-Neighbor-Interchange algorithm with search level 1 in which the initial trees were obtained with the random addition of sequences. Bayesian Inference analyses were performed with MrBayes version 3.2. (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) using the model of sequence evolution chosen by MrModeltest version 2.3 (Nylander, 2004) (For ITS1: GTR+I+G model (base frequencies A: 0.2093 / C: 0.2960 / G: 0.2884 / T: 0.2062; substitution rates A-C: 0.8642 / A-G: 1.6002 / A-T: 0.9776 / C-G: 0.5285 / C-T: 2.2255 / G-T: 1.000; among site rate variation: 0.1665; gamma distribution shape

parameter: 2.4447); for partial *rbc*L-spacer partial *rbc*S: HKY+G (same parameters as in ML model); and for COX3: GTR+I+G (base frequencies A: 0.2486 / C: 0.1609 / G: 0.1919 / T: 0.3985; substitution rates A-C: 1.4259 / A-G: 3.5364 / A-T: 1.4637 / C-G: 1.4843 / C-T: 5.8919 / G-T: 1.000; among site rate variation: 0.3350; gamma distribution shape parameter: 0.7953). Metropolis-Coupled Markov Chain Monte Carlo (MCMCMC) analyses were conducted during 4,000,000 generations with four incrementally heated chains. Four runs were conducted and each run was sampled every 1,000 generations. The default parameters were used for temperature and swapping. Convergence was checked visually by plotting likelihood vs. generations for the four runs. Therefore to ensure stability, the first 1,000 generations were discarded as the "burn-in" phase and the remaining trees were used to compute the consensus tree.

Both ITS1 trees were rooted with *Adenocystis utricularis* (GenBank accession number Z98568); for partial *rbc*L-spacer partial *rbc*S, *Ectocarpus* sp. and two sequences of *A. utricularis* were used as outgroups for ML and BI trees respectively (GenBank accession numbers: GU252552, GU252520, GU252521; respectively); and for COX3, both trees were rooted with *Pylaiella littoralis* (GenBank accession number AJ277126).

Sequences divergence, estimated as the average number of nucleotide differences between lineages or species, was estimated using DNAsp (Rozas et al., 2003).

2.5. *Morphological analysis of species within* Petalonia and Endarachne

Identification of species within *Petalonia* and *Endarachne* and their morphological variation was analyzed based on the available literature (Abbott and Hollenberg, 1976; Lee et al., 1992; Nizamuddin and Faroqui, 1968; Noda, 1969; Parente et al., 2003). Selected characters were length/width of cortical and medulla cells, and length/width of plurilocular sporangia, which were recorded and measured using cross sections obtained with a

freezing microtome and a Nikon Microscopy Unit (Corning Inc., Sturbridge, MA, USA) equipped with a digital system for image acquisition. Subsequent analyzes were done using Image Pro Plus version 4.5 software (Media Cybernetics, Silver Spring, MD, USA).

To evaluate differences among localities for each morphological species, each morphological character was tested with a one-way ANOVA (Zar, 1996) followed by a Tukey *post-hoc* test. Before testing, data were checked for homogeneity of variances. When required to fulfill the requirement of heterogeneity of variances, data were either cos-transformed or log-transformed.

Multivariate analyses were performed in PRIMER version 5 (Clarke and Gorley, 2001). A normalized Euclidean distance dissimilarity matrix was calculated from untransformed data using the full balanced data set on all morphological characters. Difference in morphology among locations was tested with an analysis of similarity (ANOSIM). Data were graphically represented in non-metric multidimensional scaling (nMDS) plots and similarity of percentages (SIMPER) analysis was used to identify the main morphological characters contributing to the observed patterns.

2.6. *Morphological analysis of* Scytosiphon *species*

Identification of species within *Scytosiphon* and their within-species and between-species morphological variations were analyzed based on the available literature (Abbott and Hollenberg, 1976; Aguilar-Rosas et al., 2006; Clayton, 1976; Hoffman and Santelices, 1997; Kogame, 1998; Kogame, 1996; Wynne, 1969). Morphological characters scored to construct a matrix for statistical analyses were length/width of cortical cells, medullar cells, plurilocular sporangia and ascocysts, presence/absence of ascocysts, type of plurilocular sporangia (loose or coherent) and external morphology (constricted, cylindrical, thin and flat). A total of 10 individuals were collected in each locality and for

each individual ca. 10 measurements of each morphometric character were recorded. Procedures for tissue processing, observations and data acquisition were as described for material belonging to *Endarachne* and *Petalonia*.

To test the significance of differences within each morphological species, each morphological variable was analyzed independently by one-way ANOVA (Zar, 1996) followed by a Tukey post-hoc test. When required to fulfill the requirement of heterogeneity of variances, data were cos-transformed

Multivariate analyses were done as described above and difference in external morphology among all sampled individuals was tested with an ANOSIM. Species assigned by morphological characters (e.g. morphological species) were used as *a priori* groups as well as species assigned by molecular characters (e.g. phylogenetic species).

3. RESULTS

3.1. *Phylogenetic analyses*

The ITS1 sequences consisted of 631 bases, the alignment presented 78 parsimony informative sites for 206 taxa and many gaps were detected on the alignment. The MP tree was recovered with a high confidence, with CI = 0.70 and RI = 0.97. The monophyly of *Scytosiphon* and *Petalonia* genus is questioned as both genera are paraphyletic and *Endarachne* appeared mixed with them (Figures 1 and 2). The *S. lomentaria* clade was subdivided in three strongly supported lineages; one lineage distributed in Chile (La Lancha, C. Palito and C. Palito canal; Figures A1 and A2) and Europe (Norway, United Kingdom, France and Greece; Figures A1 and A2), a second one composed of individuals with Pacific distribution including Chilean individuals (from Pichidangui to Fuerte Bulnes, ~ 3800 km apart; Figure A1), and the third lineage composed of individuals with Pacific distribution, which includes one individual reported from Cape Elizabeth, USA (Atlantic distribution). That is, the S. *lomentaria* clade includes a Pacific, a Pacific/Atlantic, and a Chilean/European lineage (Figures 1 and 2). In the MP tree, the three *S. lomentaria* clades were present, however the position of the Chilean/European *S. lomentaria* clade was different (Figure 2) and grouped with *S. tenellus* instead of grouping with any of the other *S. lomentaria* clades.

Sequences divergence, estimated as the average number of nucleotide differences, within each lineage were very small and ranged between 2.3 and 6.1 differences, whereas sequence variations between the Pacific and Pacific/Atlantic vs. Chilean/European lineages were high, 67.8 and 72.7 respectively. Two species of the genus *Scytosiphon* grouped with *Petalonia* species: *S. gracilis* from central Chile with *P. zosterifolia* (not found in the Chilean coast), and a clade composed of individuals from central and southern Chile only

(hereafter *Scytosiphon* sp.) grouped together with *P. fascia*, present in central and southern Chile (Figure A1). These results support the paraphyly of *Petalonia* and *Scytosiphon* (Figure 1 and 2). Another important feature to consider is the sequence divergence within each genus. *P. fascia* and *P. zosterifolia* differed in an average of 176.5 nucleotides, and average nucleotide differences within *Scytosiphon* species ranged from 44.26 between *S. lomentaria* Pacific and Pacific/Atlantic lineages to 176.41 between *S. gracilis* and *S. tenellus*. *E. binghamiae* was no exception, and although it appears as a monophyletic clade in the phylogeny (Figure 1 and 2), is part of a major clade composed by members of the other two genera considered in this study.

The partial *rbc*L-spacer and *rbc*S sequences consisted of 477bp and the alignment presented 185 constant characters and 93 parsimony informative sites for 148 taxa. MP tree had reduced homoplasy with CI and RI values of 0.70 and 0.93 respectively. A highly supported *S. lomentaria* clade became evident. As in the ITS1 tree, this clade was separated in two lineages: a Chilean/European and a Pacific/Atlantic (Figure 3 and 4). The basal species of this clade was *S. dotyi*, which has been proposed as a synonym of *S. lomentaria* (Pérez-Cirera and Cremades, 1991). As in the ITS1 phylogeny (Figure 1 and 2), paraphyly of the genus *Scytosiphon* is evident (Figure 3 and 4). In this case a moderately supported *P. fascia* clade grouped with a highly supported *S. tenellus* clade, but not with *Scytosiphon* sp. as displayed in Figure 1 and 2. Particularly, in the MP tree, *Scytosiphon* sp. clade was external to the members of the *Scytosiphon*, *Petalonia* and *Endarachne* genera (Figure 4). *E. binghamiae* grouped with *P. fascia – S. tenellus* clade, and *S. gracilis*, unlike his position in the ITS1 tree, grouped with *S. canaliculatus*. The *Colpomenia* clade, including *C. bullosa*, *C. sinuosa* and *C. peregrina*, appeared as the most basal group of the ingroup with Chilean samples (Figure 3). Interestingly, two individuals morphologically assigned to *E. binghamiae* (from Algarrobo and La Boca, Figure A3 and

A4) appeared within the *S. lomentaria* Pacific/Atlantic clade, and four individuals morphologically assigned to the *S. lomentaria* (from central Chile, Figure A3 and A4) grouped with *E. binghamiae*, based on the RUBISCO marker.

The COX3 sequences consisted of 518 bp and the alignment presented 212 constant characters and 154 parsimony informative sites for 129 taxa. MP tree had CI and RI values of 0.44 and 0.88 respectively. As for the ITS1 and partial RUBISCO markers, *S. lomentaria* appeared as a monophyletic group consisting of two lineages, the Chilean/European and the Pacific plus three sequences of the species collected on Japan (Figure 5 and 6). Again, none of the genera were monophyletic, except for *Colpomenia*. Also, the position of *S. gracilis* and *S. tenellus* differed from those displayed by the other markers. In this case, *S. gracilis* grouped with *Scytosiphon* sp. and *S. tenellus* was the most external species of the genus *Scytosiphon* (Figure 5 and 6). In the MP tree, also the position of the *S. lomentaria* Chile/European and Japanese sequences was external to *Petalonia*, *Endarachne* and *Scytosiphon*, although there was no statistical support for those nodes (Figure 6). The rest of the topology of the MP tree was similar to the ML and BI trees.

In summary, considering all the phylogenies, and independent of the molecular marker used, we identified five clades within the genus *Scytosiphon*: 1) *S. lomentaria* restricted to Europe-Chile, 2) *S. lomentaria* displaying a worldwide distribution, 3) *Scytosiphon* sp. endemic to the South coast of Chile, 4) *S. gracilis* restricted to central Chile and 5) *S. tenellus* located at one location on northern Chile. For the genus *Petalonia* we identified two clades: *P. fascia* and *P. zosterifolia*. Only the first one comprised individuals from the Southeastern Pacific coast, distributed from central (Cachagua) to southern Chile (Mar Brava) (Table 3). The genus *Endarachne* along the Chilean coast is represented only by *E. binghamiae*, distributed from northern (Arica) to central (Matanza)

Chile (Table 3). This species and *P. fascia* are almost indistinguishable in the field, but molecular classification is precise and consistent (Table 3).

3.2. *Morphological analyses of* Petalonia *and* Endarachne

Based on morphological characters, it was possible to recognize in most of the cases two morphotypes within the samples. One corresponded to what has been described as *P. fascia* and the other to *E. binghamiae*. The main difference between both morphotypes was the structure of the medulla, with round and colorless cells in *P. fascia* and interwoven colorless filaments in *E. binghamiae* (Figure 7a and b). However, in three localities of central Chile, the structure of the medulla in some samples showed both cells and filaments (Figure 7c and d). Both species presented a disjoint distribution along most of the southeastern Pacific with *P. fascia* colonizing central and southern Chile (with the exception of two localities in northern Chile) and *E. binghamiae* living in central and northern Chile (Table 3). But in some localities of central Chile (Maitencillo, Algarrobo and Las Cruces) both species co-occured.

Classification of both species (Figure 8) based on all measured morphometric characters showed a clear separation between *P. fascia* and *E. binghamiae*, which was further supported by ANOSIM that revealed high and significant differences between both species ($R = 0.932$, $P = 0.001$). For *P. fascia*, the morphological characters varied among localities; four of the six characters were significantly different (Table 4) and the classification showed that differences between localities were moderated (Figure 9a). This result was further supported by SIMPER (Table 5) which showed low percent of dissimilarity between *P. fascia* of different localities. Individuals from Maitencillo and Los Verdes were the most distinctive (24.6% dissimilarity, Table 5) and the anatomy of the medulla was the main contributor to this dissimilarity. In the case of *E. binghamiae*, all

localities but La Boca showed similar morphology (Figure 9b, Table 4). Accordingly, the individuals from La Boca showed the highest dissimilarity with the other localities (16.4–30.7%, Table 6). Size of plurilocular sporangia was the character that explained better this dissimilarity.

3.3. *Morphological analyses of* Scytosiphon *species*

Erect thalli displaying macroscopic features typically assigned to *Scytosiphon* were found in 27 localities. Four external thallus morphologies (Figure 10, Table 1) were found along its distribution, with 96.3% of the localities presenting thalli with constrictions, 29.6% cylindrical, 33.3% thin and 37% flat. Co-occurrence of at least two different external morphologies was found in 48% of the localities. Since the external morphology of erect thallus is the character most traditionally used to discriminate within *Scytosiphon* species (e.g. *S. lomentaria:* constricted; *S. tenellus:* flat; *S. gracilis:* cylindrical; Abbott and Hollenberg, 1976; Kogame, 1998; Parente et al., 2003), we run analyses to evaluate its discriminant power. The ordination revealed no apparent grouping based on external morphology (Figure 11). ANOSIM led to the same result ($R = 0.008$, $P = 0.19$) and the pairwise comparison between external morphology revealed that cylindrical erect thalli could not be distinguished from any other morphology (Table 7), demonstrating that external morphology is not a good character to discriminate among species of *Scytosiphon*. Furthermore, the morphological analyses based on internal and external characters including all samples revealed the occurrence of three discrete morphological entities along the Chilean coast: *S. gracilis*, *S. lomentaria* and *S. tenellus*. The analysis of variance of the morphological characters between species revealed that half of them (plurilocular sporangia width, cortex width, length and width of ascocysts) differed significantly (Table 4). The discriminant analysis using *a priori* groups defined by external morphology

showed that only 82.3% of the assignments were correct, with *S. gracilis* being correctly assigned most of the times (98%, Table 8), while *S. lomentaria* and *S. tenellus* showed 83% and 75% of correct assignment, respectively. Consistent with these results, the classification (Figure 12) showed no differentiation between *S. lomentaria* and *S. tenellus*, although *S. gracilis* did appear separated. Also, the ANOSIM showed no significant difference within the three species (R = 0.143, P = 0.001). However, pairwise comparisons indicated that *S. gracilis* was significantly different from *S. lomentaria* and *S. tenellus* (R = 0.565, P = 0.001 and R = 0.817, P = 0.001, respectively). The absence of ascocysts that characterizes *S. gracilis* was a key feature to separate it from the other species. Therefore, if the presence/absence of ascocysts together with the type of plurilocular sporangia (loose/coherent) are considered, the species of *Scytosiphon* can be reliably separated into three main groups (Figure 13). However, within each group the lack of morphological features to discriminate between species continues. Based on the above, an additional discriminant analysis was run with the *a priori* assignment based on the genotypic cluster found in the phylogenies. The results revealed 100% of correct assignment for *S. gracilis* and 94.1% for *S. lomentaria* (Table 9). However, erect thalli assigned to *S. tenellus* based on morphological characters (Table 8 and Figure 12) corresponded, in fact, to a taxon which does not fit within any of the currently reported species based on molecular analysis (Figure 1 to 6). By comparing the morphological characters of this entity, named *Scytosiphon* sp., with those of the other *Scytosiphon* species (Table 10), its morphological similarity with *S. tenellus* became apparent. These results suggest strongly the occurrence of a new entity that morphologically could not be separated from *S. tenellus* but molecular phylogenies could separate both entities clearly, revealing the presence of a cryptic species.

4. DISCUSSION

The results presented here for the Southeastern Pacific distribution of the Scytosiphonaceae confirm the major confusion in the current classification of the family, in view of the paraphyly of the genera characterized in this study. This view is shared by other authors (Kogame et al., 1999 and 2011; Cho et al., 2001 and 2006) who have developed molecular approaches to revisit this classification. I first discuss the observation that species of *Petalonia* and *Endarachne* are undistinguishable from those of the genus *Scytosiphon*, then I synthetize the taxonomic status of the currently recognized species of *Scytosiphon* and finally showed that the current *S. lomentaria* groups represent different entities that likely are cryptic species.

Validity of Petalonia *and* Endarachne *as separate genera*

Traditionally, solid vs. hollow thallus has been used as the main character to distinguish *Petalonia* and *Endarachne* from *Scytosiphon* (Noda et al. 1969). Nevertheless, Stegenga et al. (1997) reported that *P. zosterifolia* should be considered the compressed form of *S. lomentaria*, since the sides are closely adjacent but not fused, evidencing the weakness of hollow *versus* solid thallus as a diagnostic character. In spite of that, *P. zosterifolia* continues to be recognized as a formal species. According to several authors (Lee et al., 1992; Hoffman and Santelices, 1997; Parente et al., 2003), *E. binghamiae* is not distinguishable by naked eye from large individuals of *P. fascia*, but the microscopic structure of the fronds (medulla composed of filaments or round cells) differ. Our morphological analyses showed that the main difference between the two species is the type of medulla, and the remaining characters are almost indistinguishable. However, in some localities the alternative variants of this character appeared simultaneously in a single

individual (i.e. plants with a medulla composed of rounded cells and filaments). In summary, morphological analyses revealed that both entities were not different enough to belong to different genera. Surprisingly, the characters of the medulla described above were used recently to describe species of *Petalonia* from Hawaiian Islands (Kogame et al 2011): *P. tatewaki* presents a medulla conformed of large elongate cells and inner, intertwined rhizoidal filaments. However, it is possible to distinguish the Hawaiian *Petalonia* from *P. fascia* and *E. binghamiae* because it possesses ascocysts among plurilocular sporangia.

From a molecular point of view, they appear as sister species and forming a monophyletic group with *S. tenellus* in both ITS1 and partial RUBISCO phylogenies. With the mitochondrial marker, they appeared included within the *Scytosiphon* clade in which *S. tenellus* was more basal. According to Kogame et al. (1998), *E. binghamiae*, *P. fascia* and *S. tenellus* shared a *Stragularia* type crustose alternate phase, which it is suggested as a valid character for *Scytosiphon* genus classification. In summary, considering the above mention plus our evidence that in the three phylogenetic trees *E. binghamiae* and *P. fascia* appear within the clade of the genus *Scytosiphon* and that the morphological characters traditionally used to separate *Petalonia/Endarachne* from *Scytosiphon* are discredited, we conclude that these two genera should be re-classified within *Scytosiphon*. Further phylogenetic analyses should include a more complete species sampling within the three genera to confirm this taxonomic proposition.

Taxonomic complexity of the genus Scytosiphon *in the southeastern Pacific*

The results of the combined molecular and morphological analysis confirmed the presence of the three previously reported species, *S. lomentaria, S. tenellus* and *S. gracilis*. In addition, it revealed the presence of possibly three entities within *S. lomentaria*: two

deeply divergent sub-groups named *lomentaria* but of different geographical origins and a likely new undescribed cryptic species.

In relation to *S. gracilis* it has been reported for the coast of Japan (Kogame, 1998), Korea (Cho et al., 2002), Pacific coast of Mexico (Aguilar-Rosas et al., 2006) and recently in two distant localities in Chile (Contreras et al. 2007). One of the three ITS1 haplotypes present in Chile is also present in Korea and Japan, evidencing a common origin of these populations and a likely trans-Pacific transport of propagules. Similar finding concerned *S. tenellus* for which no erect thallus was detected along the Chilean coast (only the crustose phase was observed). This species was known for the coast of northern Japan where both erect haploid and crustose diploid phases are present (Kogame, 1998), until Camus et al. (2005) reported it from Caleta Palito, a highly contaminated site in northern Chile. Our molecular analyses showed a high similarity between samples from Chile and Japan for the ITS1, whereas four COX 3 haplotypes where recovered. It is not possible so far to infer the origin of these two species. A more comprehensive estimation of their distribution range and the spatial repartition of their genetic diversity in both Chile and Asia would be necessary.

Surprising was the finding that *S. lomentaria* is actually subdivided into 3 entities, possibly cryptic species with different but all highly disjoints distributions around the world: the Pacific, the Pacific/Atlantic and the Chilean/European. This separation was also suggested by Cho et al. (2007) and Camus et al. (2005), who proposed that each lineage was a separate species. However, they found no morphological differentiation between them, and finally suggested the occurrence of cryptic diversity. Our current results reinforce this view, but could not increase the morphological discrimination. So far, these genetically divergent groups were impossible to distinguish based on morphological characters as they present both constricted or cylindrical external morphology and the internal anatomic

features overlap. An intriguing feature of these lineages is that there are all found in Caleta Palito, a rocky beach that has been contaminated by copper mine wastes for more than 60 years (Castilla and Nealler, 1978; Correa et al., 1996). This site concentrated a total of 4 *Scytosiphon* species (including *S. tenellus*) and was therefore the most diverse of our study.

Finally, the examination of *Scytosiphon* samples revealed a monophyletic clade highly supported for each of the three molecular markers and so far reported only for Chilean coasts. This group of individuals were morphologically indistinguishable from *S. tenellus* but showed a high genetic diversity compared to any of the other *Scytosiphon* clades analyzed so far. This diversity likely indicates a Southeastern Pacific origin. The phylogenies of ITS1 and partial RUBISCO also support an affinity with *S. tenellus*. As for *Endarachne* and *Petalonia*, this affinity is not supported with the COX 3 marker for which *S. tenellus* is basal to the *Scytosiphon* clade. All together, these results tend to support the recognition of a new species, *Sytosiphon* sp., possibly endemic to the region.

5. CONCLUSION

The results presented here confirm that traditional morphometric characters for taxonomic identification and classification generally overlap among species and genera of the Scytosiphonaceae. In addition, *Endarachne*, *Petalonia* and *Scytosiphon* form a single, strongly supported clade in all our analyses. These results, combined with earlier findings from molecular studies (Kogame et al., 1999; Cho et al., 2001; 2006) provide strong evidence that these genera are not distinct evolutionary lineages and therefore should be considered as a single genus.

Particularly, within the genus *Scytosiphon*, morphology-based taxonomy also failed to identify the major delimitations between species. Only a few categorical characters allowed separation of three main groups. However, this clustering is not sufficient to assign individuals to species. This situation is not exclusive for this genus, as several groups of algae present intra-generic taxonomic problems, including the green genera *Enteromorpha*/*Ulva* (Blomster et al., 1998), the brown genus *Sargassum* (Cheang et al., 2008) and the siphonocladalean *Boodlea* complex (Leliaert et al., 2009). An increasing number of cryptic species are being reported since the development of molecular markers and barcoding approaches in algae (see Saunders and LeGall 2010, and the associated Special Issue of *Cryptogamie Algologie*). In particular, these studies tend to reveal that supposedly cosmopolitan species may be in fact a number of geographically restricted cryptic species (Le Gall and Saunders 2010). Interestingly, what was considered so far as *S. lomentaria* is cosmopolitan and subdivided into different cryptic species, each of which seem to be also widely distributed. Recent introductions from source populations likely occurred in most cases. Future studies to resolve the evolutionary relationship between

species and the origin of diversification of the genus should include *ad hoc* sampling of the intra-specific diversity and characterization of their respective distribution range.

6. ACKNOWLEDGMENTS

This research was funded by FONDAP 1501-0001 (CONICYT) and the Center for Advanced Studies in Ecology and Biodiversity (CASEB) Program 7. C.Camus gratitude CONICYT for her doctoral grant and sincerely thank Jessica Beltrán, Martha Hengst and Alejandra Gonzalez for collecting specimens and assisting in the field and Veronica Flores for helping with anatomical analyses.

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TABLE 1: Collection sites and GenBank accesion numbers for species used in this study. External morphology for *Scytosiphon* erect thallus only.

Collection site and date	Abbreviation	Coordinates (Latitude, Longitude)	GenBank accesion number			External morphology
			Nuclear	Chloroplastidial	Mitochondrial	
Scytosiphon spp.						
Piquero; 27 May 2006	PI		GU252376		GU252592	Cylindrical
Piquero; 27 May 2006	PI				GU252570	Constriction
Caleta Palito 200; 14 July 2007	CP	26º15,79´S; 70º40,63´W	GU252410	GU252402	GU252561	Constriction
Caleta Palito canal; 14 July 2007	CPC	26º15,79´S; 70º40,63´W	GU252413	GU252403	GU252560	Constriction
La Lancha; 13 July 2007	LL	26º13,19´S; 70º39,83´W	GU252411	GU252404	GU252563	Cylindrical
La Lancha; 13 July 2007	LL	26º13,19´S; 70º39,83´W	GU252412	GU252405	GU252562	Constriction
Coquimbo; 30 July 2007	COQ	29°56,108´S; 71°20,168´W	GU252382	GU252407	GU252569	Constriction
Coquimbo; 30 July 2007	COQ	29°56,108´S; 71°20,168´W	GU252375	GU252408	GU252591	Cylindrical
Coquimbo; 30 July 2007	COQ	29°56,108´S; 71°20,168´W		GU252494	GU252622	Flat
Pichidangui; 29 July 2007	PI	32°09,462´S; 71°31,89´W	GU252402	GU252406	GU252568	Constriction
Pichidangui; 29 July 2007	PI	32°09,462´S; 71°31,89´W	GU252396		GU252600	Cylindrical
Cachagua; 15 September 2005	CACH	32°35,058´S; 71°27,155´W	GU252380	GU252472	GU252573	Thin
Cachagua; 15 September 2005	CACH	32°35,058´S; 71°27,155´W	GU252406	GU252471	GU252574	Constriction
Cachagua; 15 September 2005	CACH	32°35,058´S; 71°27,155´W		GU252486	GU252623	Flat
Cachagua	CACH	32°35,058´S; 71°27,155´W		GU252468		
Cachagua	CACH	32°35,058´S; 71°27,155´W		GU252469		
Cachagua	CACH	32°35,058´S; 71°27,155´W		GU252470		
Maitencillo; 15 September 2005	MAI	32°39,319´S; 71°26,642´W	GU252424		GU252619	Thin
Maitencillo; 15 September 2005	MAI	32°39,319´S; 71°26,642´W	GU252404	GU252477	GU252575	Constriction
Maitencillo; 15 September 2005	MAI	32°39,319´S; 71°26,642´W	GU252381	GU252473	GU252579	Constriction
Maitencillo; 5 November 2007	MAI	32°39,319´S; 71°26,642´W	GU252418			Constriction
Maitencillo; 5 November 2007	MAI	32°39,319´S; 71°26,642´W		GU252515		Thin
Maitencillo; 5 November 2007	MAI	32°39,319´S; 71°26,642´W	GU252391		GU252602	Constriction
Reñaca; 18 October 2005	RE	32°57,326´S; 71°32,855´W	GU252398	GU252481	GU252585	Constriction
Reñaca; 18 October 2005	RE	32°57,326´S; 71°32,855´W	GU252417	GU252485	GU252638	Thin
La Boca; 18 October 2005	BOCA	32°55,083´S; 71°31,027´W	GU252399		GU252586	Constriction
Algarrobo; 28 September 2005	ALG	33°21,404´S; 71°39,606´W	GU252405	GU252484	GU252580	Constriction
Algarrobo; 28 September 2005	ALG	33°21,404´S; 71°39,606´W	GU252397	GU252480	GU252581	Cylindrical
El Quisco; 28 September 2005	QUI	33°24,060´S; 71°41,973´W	GU252400		GU252582	Constriction
El Quisco; 28 September 2005	QUI	33°24,060´S; 71°41,973´W	GU252389	GU252482	GU252583	Cylindrical
El Tabo; 28 September 2005	TABO	33°27,562´S; 71°39,810´W	GU252384		GU252584	Constriction
El Tabo; 28 September 2005	TABO	33°27,562´S; 71°39,810´W	GU252415	GU252490	GU252606	Thin
Las Cruces; 1 September 2005	LC	33°30,165´S; 71°37,976´W	GU252420	GU252475	GU252604	Thin
Las Cruces; 1 September 2005	LC	33°30,165´S; 71°37,976´W	GU252409	GU252488	GU252572	Constriction
Las Cruces; 1 September 2005	LC	33°30,165´S; 71°37,976´W		GU252476	GU252618	Cylindrical
Las Cruces; 2005	LC	33°30,165´S; 71°37,976´W	GU252414			Cylindrical

Las Cruces; 2005	LC	33°30,165´S; 71°37,976´W		GU252460		
Las Cruces; 2005	LC	33°30,165´S; 71°37,976´W		GU252461		
Pelancura; 3 November 2005	PE	33°33,420´S; 71°37,595´W	GU252429	GU252483	GU252605	Thin
Pelancura; 3 November 2005	PE	33°33,420´S; 71°37,595´W	GU252422	GU252489	GU252603	Flat
Pelancura; 3 November 2005	PE	33°33,420´S; 71°37,595´W	GU252388	GU252479	GU252566	Constriction
Pelancura; 3 November 2005	PE	33°33,420´S; 71°37,595´W	GU252408	GU252518	GU252576	Cylindrical
Pelancura; 2005	PE	33°33,420´S; 71°37,595´W		GU252462		
Pelancura; 2005	PE	33°33,420´S; 71°37,595´W		GU252463		
Pelancura; 2005	PE	33°33,420´S; 71°37,595´W		GU252464		
Pelancura; 2005	PE	33°33,420´S; 71°37,595´W		GU252465		
Pelancura; 2005	PE	33°33,420´S; 71°37,595´W		GU252466		
Pelancura; 2005	PE	33°33,420´S; 71°37,595´W		GU252467		
Matanza; 27 October 2005	MZ	33°57,736´S; 71°52,715´W	GU252386	GU252487	GU252587	Constriction
Matanza; 27 October 2005	MZ	33°57,736´S; 71°52,715´W	GU252423	GU252517	GU252609	Thin
Curanipe; 26 October 2007	CUR	35°50´30,0´´S; 72°38´20,7´´W	GU252377	GU252511	GU252595	Constriction
Cocholgue; 25 October 2007	COCH	36°36´26,4´´S; 72°58´48,9´´W	GU252430		GU252616	Flat
Cocholgue; 25 October 2007	COCH	36°36´26,4´´S; 72°58´48,9´´W	GU252394	GU252509	GU252594	Constriction
Lebu; 24 October 2007	LEBU	37°34´47,3´´S; 73°38´32,0´´W	GU252395		GU252601	Flat
Lebu; 24 October 2007	LEBU	37°34´47,3´´S; 73°38´32,0´´W	GU252385		GU252596	Constriction
Niebla; 23 October 2007	NIE	39°52,465´S; 73°24,017´W	GU252421		GU252621	Flat
Niebla; 2 March 2006	NIE	39°52,465´S; 73°24,017´W	GU252387	GU252491	GU252611	Flat
Niebla; 2 March 2006	NIE	39°52,465´S; 73°24,017´W	GU252416	GU252513	GU252612	Thin
Niebla; 2005	NIE	39°52,465´S; 73°24,017´W		GU252455		
Niebla; 2005	NIE	39°52,465´S; 73°24,017´W		GU252456		
Niebla; 2005	NIE	39°52,465´S; 73°24,017´W		GU252457		
Niebla; 2005	NIE	39°52,465´S; 73°24,017´W		GU252458		
Niebla; 2005	NIE	39°52,465´S; 73°24,017´W		GU252459		
Pucatrihue; 1 March 2006	PUCA	40°32,812´S; 73°43,150´W	GU252401	GU252495	GU252565	Constriction
Pucatrihue; 1 March 2006	PUCA	40°32,812´S; 73°43,150´W	GU252425	GU242574	GU252607	Flat
Pucatrihue; 22 October 2007	PUCA	40°32,812´S; 73°43,150´W	GU252419		GU252614	Flat
Punta Estaquilla; 1 March 2006	ESTA	41°23,644´S; 73°50,159´W	GU252403	GU252519	GU252577	Constriction
Punta Estaquilla; 1 March 2006	ESTA	41°23,644´S; 73°50,159´W	GU252428	GU252478	GU252610	Flat
Carelmapu; 28 February 2006	CAREL	41°44,465´S; 73°44,113´W	GU252392	GU252493	GU252578	Constriction
Carelmapu; 28 February 2006	CAREL	41°44,465´S; 73°44,113´W	GU252427	GU252512	GU252613	Flat
Carelmapu; 21 October 2007	CAREL	41°44,465´S; 73°44,113´W		GU252514	GU252617	Constriction
Carelmapu; 21 October 2007	CAREL	41°44,465´S; 73°44,113´W	GU252378	GU252510	GU252593	Thin
Carelmapu; 21 October 2007	CAREL	41°44,465´S; 73°44,113´W	GU252426		GU252620	Flat
Mar Brava; 28 February 2006	MB	41°52,078´S; 74°01,254´W	GU252379	GU252496	GU252598	Constriction
Mar Brava; 28 February 2006	MB	41°52,078´S; 74°01,254´W			GU252608	Flat
Mar Brava; 28 February 2006	MB	41°52,078´S; 74°01,254´W	GU252393	GU252497	GU252589	Constriction
Mar Brava; 28 February 2006	MB	41°52,078´S; 74°01,254´W	GU252373	GU252498	GU252588	Cylindrical
Mar Brava; 28 February 2006	MB	41°52,078´S; 74°01,254´W	GU252407	GU252499	GU252590	Constriction
Cucao; 2006	CU	42º38.00´´S; 74º06.00´W	GU252374	GU252516	GU252571	Constriction

Cucao; 2006	CU	42º38.00´´S; 74º06.00´W	GU252372		GU252599	Constriction
Fuerte Bulnes; 2006	FB	53°35,00´S; 73°41,00´W	GU252383	GU252492	GU252564	Constriction
Isla Carlos III; 2006	IC		GU252390	GU252500	GU252597	Constriction
Isla Carlos III; 2006	IC			GU252501	GU252567	Constriction
		Total *Scytosiphon* spp.	60	65	65	
Colpomenia spp.						
Arica; 30 August 2007	AR	18º29´39,01´´S; 70º19´36,01´´W			GU252656	
Arica; 30 August 2007	AR	18º29´39,01´´S; 70º19´36,01´´W			GU252659	
Playa Los Verdes; 29 August 2007	VE	20º25,600´S; 70º09,856´´W			GU252657	
Caleta Chipana; 29 August 2007	CHI	21º20,414´S; 70º05,753´W			GU252675	
Caleta Chipana; 29 August 2007	CHI	21º20,414´S; 70º05,753´W			GU252671	
Caleta Constitución; 10 August 2007	CONS	23°25,089´S; 70°35,503´W			GU252674	
Caleta Constitución; 10 August 2007	CONS	23°25,089´S; 70°35,503´W			GU252672	
Punta Choros; 31 July 2007	CHORO	29°14,985´S; 71°27,854´W			GU252655	
Punta Choros; 31 July 2007	CHORO	29°14,985´S; 71°27,854´W			GU252662	
Punta Choros; 31 July 2007	CHORO	29°14,985´S; 71°27,854´W			GU252666	
Coquimbo; 30 July 2007	COQ	29°56,108´S; 71°20,168´W			GU252673	
Coquimbo; 30 July 2007	COQ	29°56,108´S; 71°20,168´W			GU252663	
Pichidangui; 20 July 2007	PI	32°09,462´S; 71°31,89´W			GU252668	
Pichidangui; 20 July 2007	PI	32°09,462´S; 71°31,89´W			GU252665	
El Quisco; 28 July 2007	QUI	33°24,060´S; 71°41,973´W		GU252545	GU25253	
El Quisco; 28 July 2007	QUI	33°24,060´S; 71°41,973´W		GU252546	GU252661	
El Quisco; 28 July 2007	QUI	33°24,060´S; 71°41,973´W			GU252667	
El Tabo; 28 July 2007	TABO	33°27,562´S; 71°39,810´W		GU252550	GU252664	
El Tabo; 28 July 2007	TABO	33°27,562´S; 71°39,810´W		GU252551	GU252660	
Las Cruces; 28 July 2007	LC	33°30,165´S; 71°37,976´W		GU252547	GU252658	
Las Cruces; 28 July 2007	LC	33°30,165´S; 71°37,976´W		GU252548	GU252654	
Las Cruces; 28 July 2007	LC	33°30,165´S; 71°37,976´W		GU252549	GU252652	
Pelancura; 7 November 2007	PE	33°33,420´S; 71°37,595´W			GU252669	
Pucatrihue; 22 October 2007	PUCA	40°32,812´S; 73°43,150´W			GU252670	
		Total *Colpomenia* spp.		7	24	
Petalonia.spp/*Endarachne* spp.						
Arica; 30 August 2007	AR	18º29´39,01´´S; 70º19´36,01´´W	GU252452	GU252543	GU252625	
Playa Los Verdes; 29 August 2007	VE	20º25,600´S; 70º09,856´´W	GU252444		GU252629	
Caleta Chipana; 29 August 2007	CHI	21º20,414´S; 70º05,753´W	GU252448	GU252538	GU252632	
Caleta Cobija; 11 August 2007	COB	22°33,050´S; 70°16,052´W	GU252450	GU252541	GU252631	
Caleta Constitucion; 10 August 2007	CONS	23°25,089´S; 70°35,503´W	GU252453	GU252533	GU252635	
Paposo; 12 August 2007	PAP	25°06,858´S; 70°29,191´W	GU252451	GU252535	GU252624	
Punta Choros; 31 July 2007	CHORO	29°14,985´S; 71°27,854´W	GU252447		GU252637	
Coquimbo; 30 July 2007	COQ	29°56,108´S; 71°20,168´W	GU252449	GU252537	GU252636	
Cachagua; 15 September 2005	CACH	32°35,058´S; 71°27,155´W		GU252528	GU252634	
Cachagua	CACH	32°35,058´S; 71°27,155´W	GU252436	GU252531	GU252639	
Maitencillo; 15 September 2005	MAI	32°39,319´S; 71°26,642´W	GU252434	GU252524	GU252640	

La Boca; 18 October 2005	BOCA	32°55,083´S; 71°31,027´W		GU252526	GU252615	
Reñaca; 18 October 2005	RE	32°57,326´S; 71°32,855´W	GU252446		GU252626	
Algarrobo; 28 September 2005	ALG	33°21,404´S; 71°39,606´W	GU252445	GU252525	GU252630	
Las Cruces; 28 July 2007	LC	33°30,165´S; 71°37,976´W	GU252431	GU252536	GU252648	
Las Cruces; 28 July 2007	LC	33°30,165´S; 71°37,976´W	GU252441	GU252534	GU252644	
Las Cruces; 1 September 2005	LC	33°30,165´S; 71°37,976´W	GU252454	GU252532	GU252633	
Pelancura; 3 November 2005	PE	33°33,420´S; 71°37,595´W	GU252442	GU252540	GU252628	
Matanza; 27 October 2005	MZ	33°57,736´S; 71°52,715´W	GU252443	GU252522	GU252627	
Cocholgue; 25 October 2007	COCH	36°36´26,4´´S; 72°58´48,9´´W	GU252432	GU252548	GU252650	
Lebu; 24 October 2007	LEBU	37°34´47,3´´S; 73°38´32,0´´W	GU252433	GU252523	GU252651	
Pucatrihue; 22 October 2007	PUCA	40°32,812´S; 73°43,150´W	GU252440	GU252529	GU252646	
Pucatrihue; 1 March 2006	PUCA	40°32,812´S; 73°43,150´W	GU252439	GU252539	GU252642	
Punta Estaquilla; 1 March 2006	ESTA	41°23,644´S; 73°50,159´W		GU252527	GU252649	
Carelmapu; 28 February 2006	CAREL	41°44,465´S; 73°44,113´W	GU252435		GU252645	
Carelmapu; 21 October 2007	CAREL	41°44,465´S; 73°44,113´W	GU252438	GU252542	GU252642	
Carelmapu; 21 October 2007	CAREL	41°44,465´S; 73°44,113´W	GU252437		GU252647	
Mar Brava; 28 February 2006	MB	41°52,078´S; 74°01,254´W		GU252530	GU252641	
		Total *Petalonia/Endarachne*	25	23	28	
		Total sequences	85	95	117	**297**

TABLE 2: GenBank accesion number of published sequences used in this study.

Taxa		GenBank accesion number			Reference
		COX3	ITS1	partial *rbc*L-spacer partial *rbc*S	
S. lomentaria	Oshoro, Japan	AB094194			Kogame et al 2005
S. lomentaria	Oshoro, Japan	AB094195			Kogame et al 2005
S. lomentaria	Asari, Japan	AB094196			Kogame et al 2005
S. lomentaria	Oshoro, Japan	AB094197			Kogame et al 2005
S. lomentaria	Melbourne, Australia		AB265596	AB265691	Cho et al 2007
S. lomentaria	Melbourne, Australia		AB265597	AB265692	Cho et al 2007
S. lomentaria	Melbourne, Australia		AB265598		Cho et al 2007
S. lomentaria	Sydney, Australia		AB265599	AB265693	Cho et al 2007
S. lomentaria	Akkeshi, Japan		AB265600	AB265694	Cho et al 2007
S. lomentaria	Kannonzaki, Japan		AB265601		Cho et al 2007
S. lomentaria	Muroran, Japan		AB265602	AB265695	Cho et al 2007
S. lomentaria	Muroran, Japan		AB265603		Cho et al 2007
S. lomentaria	Muroran, Japan		AB265604	AB265696	Cho et al 2007
S. lomentaria	Muroran, Japan		AB265605		Cho et al 2007
S. lomentaria	Oshoro, Japan		AB265606		Cho et al 2007
S. lomentaria	Shikanoshima, Japan		AB265607		Cho et al 2007
S. lomentaria	Shimoda, Japan		AB265608		Cho et al 2007
S. lomentaria	Andeok, Korea		AB265609		Cho et al 2007
S. lomentaria	Andeok, Korea		AB265610	AB265699	Cho et al 2007
S. lomentaria	Andeok, Korea		AB265611		Cho et al 2007
S. lomentaria	Anin, Korea		AB265612		Cho et al 2007
S. lomentaria	Bigeumdo, Korea		AB265613	AB265701	Cho et al 2007
S. lomentaria	Gyeokpo, Korea		AB265614		Cho et al 2007
S. lomentaria	Gyeokpo, Korea		AB265615	AB265703	Cho et al 2007
S. lomentaria	Cheonjin, Korea		AB265616	AB265704	Cho et al 2007
S. lomentaria	Cheonjin, Korea		AB265617		Cho et al 2007
S. lomentaria	Daecheon, Korea		AB265618	AB265705	Cho et al 2007
S. lomentaria	Gampo, Korea		AB265619	AB265706	Cho et al 2007
S. lomentaria	Gampo, Korea		AB265620		Cho et al 2007
S. lomentaria	Geojedo Island, Korea		AB265621	AB265707	Cho et al 2007
S. lomentaria	Gotdo, Korea		AB265622	AB265708	Cho et al 2007
S. lomentaria	Guryongpo, Korea		AB265623		Cho et al 2007
S. lomentaria	Guryongpo, Korea		AB265624		Cho et al 2007
S. lomentaria	Hado, Korea		AB265625		Cho et al 2007
S. lomentaria	Hallim, Korea		AB265626	AB265710	Cho et al 2007
S. lomentaria	Jindo, Korea		AB265627		Cho et al 2007
S. lomentaria	Jindo, Korea		AB265628	AB265712	Cho et al 2007
S. lomentaria	Hupo, Korea		AB265629	AB265713	Cho et al 2007

S. lomentaria	Jangho, Korea	AB265630	AB265714	Cho et al 2007
S. lomentaria	Wando, Korea	AB265631	AB265715	Cho et al 2007
S. lomentaria	Lachido, Korea	AB265632	AB265716	Cho et al 2007
S. lomentaria	Namhaedo, Korea	AB265633		Cho et al 2007
S. lomentaria	Namhaedo, Korea	AB265634		Cho et al 2007
S. lomentaria	Oeyeondo, Korea	AB265635	AB265717	Cho et al 2007
S. lomentaria	Sacheon, Korea	AB265636	AB265718	Cho et al 2007
S. lomentaria	Sacheon, Korea		AB265719	Cho et al 2007
S. lomentaria	Sacheon, Korea	AB265637		Cho et al 2007
S. lomentaria	Chejudo, Korea	AB265640		Cho et al 2007
S. lomentaria	Songjeong, Korea	AB265641		Cho et al 2007
S. lomentaria	Sacheon, Korea	AB265638	AB265720	Cho et al 2007
S. lomentaria	Sacheon, Korea	AB265639	AB265721	Cho et al 2007
S. lomentaria	Seongsan, Korea		AB265722	Cho et al 2007
S. lomentaria	Songtando, Korea	AB265642	AB265723	Cho et al 2007
S. lomentaria	Woongdo, Korea	AB265643	AB265724	Cho et al 2007
S. lomentaria	Dunedin, New Zealand	AB265644		Cho et al 2007
S. lomentaria	Dunedin, New Zealand	AB265645		Cho et al 2007
S. lomentaria	Dunedin, New Zealand	AB265646		Cho et al 2007
S. lomentaria	Dunedin, New Zealand	AB265647		Cho et al 2007
S. lomentaria	Wellington, New Zealand	AB265648		Cho et al 2007
S. lomentaria	Wellington, New Zealand	AB265649		Cho et al 2007
S. lomentaria	Wellington, New Zealand	AB265650		Cho et al 2007
S. lomentaria	Wellington, New Zealand	AB265651		Cho et al 2007
S. lomentaria	Dunedin, New Zealand	AB265652		Cho et al 2007
S. lomentaria	Wellington, New Zealand	AB265653		Cho et al 2007
S. lomentaria	Wellington, New Zealand	AB265654		Cho et al 2007
S. lomentaria	Dunedin, New Zealand	AB265655		Cho et al 2007
S. lomentaria	Dunedin, New Zealand	AB265656		Cho et al 2007
S. lomentaria	Oregon, USA	AB265657	AB265726	Cho et al 2007
S. lomentaria	Oregon, USA	AB265658		Cho et al 2007
S. lomentaria	Oregon, USA	AB265659		Cho et al 2007
S. lomentaria	Oregon, USA	AB265660		Cho et al 2007
S. lomentaria	Oregon, USA	AB265661		Cho et al 2007
S. lomentaria	Maine, USA	AB265662	AB265727	Cho et al 2007
S. lomentaria	Oregon, USA	AB265663		Cho et al 2007
S. lomentaria	Oregon, USA	AB265664		Cho et al 2007
S. lomentaria	Oregon, USA	AB265665		Cho et al 2007
S. lomentaria	Oregon, USA	AB265666		Cho et al 2007
S. lomentaria	Washington, USA	AB562667	AB265728	Cho et al 2007
S. lomentaria	Washington, USA	AB265668		Cho et al 2007
S. lomentaria	Washington, USA	AB265669		Cho et al 2007
S. lomentaria	Kamchatka, Russia	AB265670	AB265729	Cho et al 2007

S. lomentaria	Roscoff, France	AB265671	AB265730	Cho et al 2007
S. lomentaria	Roscoff, France	AB265672	AB265731	Cho et al 2007
S. lomentaria	Roscoff, France	AB265673		Cho et al 2007
S. lomentaria	Bergen, Norway	AB265674	AB265732	Cho et al 2007
S. lomentaria	Isle of Man, UK	AB265675	AB265733	Cho et al 2007
S. lomentaria	Isle of Man, UK	AB265676		Cho et al 2007
S. lomentaria	Caleta Palito, Chile		DQ151616	Camus et al 2005
S. lomentaria	Caleta Palito, Chile		DQ151617	Camus et al 2005
S. lomentaria	Caleta Palito, Chile		DQ151618	Camus et al 2005
S. lomentaria	Caleta Palito, Chile		DQ151619	Camus et al 2005
S. lomentaria	Laguna, Chile	DQ916026		Contreras et al 2007
S. lomentaria	Maitencillo, Chile	DQ916025		Contreras et al 2007
S. lomentaria	Chañaral, Chile	DQ916024		Contreras et al 2007
S. lomentaria	Chañaral, Chile	DQ916023		Contreras et al 2007
S. lomentaria	Athens, Greece	AB195216		Camus et al 2005
S. lomentaria	Oshoro, Japan	AB195215		Camus et al 2005
S. tenellus	Muroran, Japan	AB195214		Camus et al 2005
S. tenellus	Caleta Palito, Chile	DQ151634	DQ151625	Camus et al 2005
S. tenellus	Caleta Palito, Chile	DQ151635	DQ151624	Camus et al 2005
S. tenellus	Caleta Palito, Chile	DQ151633	DQ151623	Camus et al 2005
S. tenellus	Caleta Palito, Chile	DQ151632	DQ151622	Camus et al 2005
S. tenellus	La Lancha, Chile	DQ151631	DQ151621	Camus et al 2005
S. tenellus	Caleta Palito, Chile	DQ151630	DQ151620	Camus et al 2005
S. gracilis	Laguna, Chile	DQ916035		Contreras et al 2007
S. gracilis	Maitencillo, Chile	DQ916034		Contreras et al 2007
S. gracilis	Cachagua, Chile	DQ916033		Contreras et al 2007
S. gracilis	Korea, Hado	AY154740		Cho et al 2002
S. gracilis	Korea, Hado	AY154739		Cho et al 2002
S. gracilis	Hado, Korea		AF385852	Cho et al 2001
S. canaliculatus	Oshoro, Japan		AB195212	Camus et al 2005
S. dotyi	California, USA		AF385851	Cho et al 2001
P. fascia	Kamchatka, Russia		AF385844	Cho et al 2001
P. fascia	Kamchatka, Russia		AF385845	Cho et al 2001
P. fascia	Awajii, Japan		AF385846	Cho et al 2001
P. fascia	Guryongpo, Korea		AF385847	Cho et al 2001
P. fascia	Oregon, USA		AF385848	Cho et al 2001
P. fascia	Roscoff, France		AF385849	Cho et al 2001
P. fascia	Wando, Korea		AF385850	Cho et al 2001
P. binghamiae	Jeju, Korea		AF385840	Cho et al 2001
P. binghamiae	Sacheon, Korea		AF385841	Cho et al 2001
P. binghamiae	Shimoda, Japan		AF385842	Cho et al 2001
P. binghamiae	California, USA		AF385843	Cho et al 2001
C. bullosa	Guryongpo, Korea		AF385835	Cho et al 2001

C. bullosa	Jeju, Korea	AF385836	Cho et al 2001
C.peregrina	Seocheon, Korea	AF385837	Cho et al 2001
C. peregrina	Roscoff, France	AF385838	Cho et al 2001
C. sinuosa	Jeju, Korea	AF385839	Cho et al 2001

TABLE 3: Morphological and molecular classification of *P. fascia* and *E. binghamiae* collected along the Southeastern Pacific coast. Abbreviation of sites as in TABLE 1.

Collection site	Classification			
	Morphological	Molecular		
		partial COX3	partial LSU-ITS1 partial SSU	partial *rbc*L-spacer partial *rbc*S
AR	*P. fascia*	*E. binghamiae*	*E. binghamiae*	*E. binghamiae*
VE	*P. fascia*	*E. binghamiae*	*E. binghamiae*	
CHI	*E. binghamiae*	*E. binghamiae*	*E. binghamiae*	*E. binghamiae*
COB	*E. binghamiae*	*E. binghamiae*	*E. binghamiae*	*E. binghamiae*
CONS	*E. binghamiae*	*E. binghamiae*	*E. binghamiae*	*E. binghamiae*
PAP	*E. binghamiae*	*E. binghamiae*	*E. binghamiae*	*E. binghamiae*
CAR	*E. binghamiae*			
CHOROS	*E. binghamiae*	*E. binghamiae*	*E. binghamiae*	
COQ	*E. binghamiae*	*E. binghamiae*	*E. binghamiae*	*E. binghamiae*
CACH	*E. binghamiae*	*E. binghamiae/P. fascia*	*P.fascia*	*E. binghamiae / P. fascia*
MAIT	*E. binghamiae / P. fascia*	*P. fascia*	*E. binghamiae / P. fascia*	*P. fascia*
BOCA	*E. binghamiae*	*E. binghamiae*		
RE	*E. binghamiae*	*E. binghamiae*		
ALG	*E. binghamiae / P. fascia*	*E. binghamiae*	*E. binghamiae*	
LC	*E. binghamiae / P. fascia*	*E. binghamiae / P. fascia*	*E. binghamiae / P. fascia*	*E. binghamiae / P. fascia*
PE	*E. binghamiae*	*E. binghamiae*	*E. binghamiae / P. fascia*	*E. binghamiae*
MZ	*E. binghamiae*	*E. binghamiae*	*E. binghamiae*	*E. binghamiae*
COCH	*P. fascia*	*P. fascia*	*P. fascia*	*P. fascia*
LEBU	*P. fascia*	*P. fascia*	*P. fascia*	*P. fascia*
PUC	*P. fascia*	*P. fascia*	*P. fascia*	*P. fascia*
ESTA	*P. fascia*	*P. fascia*		*P. fascia*
CAREL	*P. fascia*	*P. fascia*	*P. fascia*	*P. fascia*
MB	*P. fascia*	*P. fascia*		*P. fascia*

TABLE 4: Results from ANOVA on individual morphological characters for *P. fascia*, *E. binghamiae* and *Scytosiphon* species.

Morphological character	*F*	*P*
P. fascia		
Plurilocular sporangia length	0.99	0.449
Plurilocular sporangia width	72.01	0.000*
Cortex length	2.97	0.002*
Cortex width	2.46	0.010*
Medulla length	2.34	0.011*
Medulla width	0.92	0.515
E. binghamiae		
Plurilocular sporangia length	0.86	0.607
Plurilocular sporangia width	59.15	0.000*
Cortex length	3.09	0.000*
Cortex width	2.50	0.002*
Scytosiphon species		
Plurilocular sporangia length	0.42	0.656
Plurilocular sporangia width	9.58	0.000*
Cortex length	1.11	0.328
Cortex width	17.98	0.000*
Medulla length	0.18	0.839
Medulla width	1.97	0.140
Ascocysts length	173.19	0.000*
Ascocysts width	220.05	0.000*

TABLE5: Similarity percentage (SIMPER) table of morphological character of *P. fascia*. (Abbreviations of sites as in TABLE 1).

Localities	VE	MAIT	ALG	LC	COCH	LEBU	PUCA	ESTA	CAREL	MB
AR	13.85	10.15	12.42	10.41	8.42	9.96	8.61	9.61	9.46	12.53
VE	0.00	24.63	13.51	16.85	14.04	11.44	16.49	8.27	13.92	16.21
MAIT		0.00	14.57	13.30	12.39	18.64	9.18	19.17	13.32	14.63
ALG			0.00	5.79	8.51	14.26	8.78	6.54	6.86	2.90
LC				0.00	11.78	17.01	13.65	8.58	10.67	5.28
COCH					0.00	9.36	4.33	10.49	6.58	10.84
LEBU						0.00	12.62	9.75	11.79	16.88
PUCA							0.00	12.43	6.69	11.38
ESTA								0.00	8.76	8.50
CAREL									0.00	8.52
MB										0.00

TABLE 6: Similarity percentage (SIMPER) table of morphological character of *E. binghamiae*. (Abbreviations of sites as in TABLE 1).

Localities	COB	CONS	PAP	CHOROS	COQ	CACH	MAIT	BOCA	CO	ALG	LC	PE	MZ
CHI	14.46	11.52	17.77	6.35	9.82	9.69	9.54	16.41	11.65	7.87	3.04	16.64	22.90
COB	0.00	3.50	5.70	8.66	5.90	7.67	9.23	28.81	4.56	9.34	15.78	4.15	11.11
CONS		0.00	7.00	6.24	2.96	4.92	6.94	26.59	1.04	7.19	13.60	5.90	12.34
PAP			0.00	13.08	8.07	11.96	11.11	30.74	7.58	14.19	20.20	2.91	6.59
CHOROS				0.00	4.86	3.62	4.79	22.52	6.36	1.53	8.52	11.98	18.41
COQ					0.00	3.52	4.53	24.32	2.71	5.77	12.65	7.06	13.85
CACH						0.00	5.98	25.69	4.29	3.11	11.41	10.84	17.45
MAIT							0.00	22.45	6.75	5.65	11.45	8.65	15.76
BOCA								0.00	26.41	23.97	14.75	29.23	35.91
CO									0.00	6.57	13.25	6.52	13.35
ALG										0.00	9.56	13.04	19.66
LC											0.00	18.58	25.31
PE												0.00	7.46
MZ													0.00

TABLE 7: Analysis of Similarities (ANOSIM) of external morphology considering *Scytosiphon* erect thallus external morphology. C: constricted, F: flat, T: thin and Cy: cylindrical erect thallus.

Groups	R statistic	% significance level
C, T	0.002	0.428
C, F	0.009	0.197
C, Cy	0.035	0.027*
T, F	-0.01	0.809
T, Cy	0.093	0.001*
F, Cy	0.057	0.001*

TABLE 8: Discriminant Analysis. Species assigned by morphological characters (e.g. morphological species) were used as *a prior* groups. (N=2600; N correct=2140; Percentage=82.31%; Wilk´s lambda=0.18062 F(30,7594)=200,36 P<0.0000).

	S. gracilis	*S. lomentaria*	*S. tenellus*
S. gracilis	200	0	0
S. lomentaria	0	1430	170
S. tenellus	4	286	510
N total	204	1716	680
N correct	200	1430	510
Percentage	98.04	83.33	75

TABLE 9: Discriminant Analysis. Species assigned based on genotypic groups found on the phylogenetic trees were used as *a priori* groups. (N=2600; N correct=2450; Percentage=94.2%; Wilk´s lambda=0.06 F(22.5174)=722.2332 P<0.0000).

	S. gracilis	*Scytosiphon* sp.	*S. lomentaria*
S. gracilis	100	50	50
Scytosiphon sp.	0	750	50
S. lomentaria	0	0	1600
N total	100	800	1700
N correct	100	750	1600
Percentage	100	93.7	94.1

TABLE 10: Comparison of taxonomic characters of seven Scytosiphon species with *Scytosiphon* sp.

	Scytosiphon sp.	*S. complanatus*	*S.crispus*	*S.dotyi*	*S.gracilis*	*S.lomentaria*	*S. tenellus*
External morphology	Flat or thin	Flat	Slightly compressed	Cylindrical to flat	Cylindrical to flat	Cylindrical with constrictions	Flat
Length (cm)	2-18	Up to 50	Up to 1	Up to 12	10-25 (40)	10-50	3-15 (25)
Wide (mm)	0.6-2.2	Up to 4.5	Up to 0.3	Up to 1	1-4 (8)	3-8	1-5
Thickness of medullary layer	2-3 cells	2-3 cells	2-3 cells	2-3 cells	2-3 cells	3-5 cells	2-3 cells
Thickness of cortical layer	1-3 cells	?	?	?	1-3 cells	2-3 cells	1-3 cells
Plurilocular sporangia type	Coherent	Coherent	Coherent	Coherent	Coherent	Loose	Coherent
Ascocyst	Present	Absent	Absent	Absent	Absent	Present	Present

Data from: Kogame (1996); Wynne (1969); Abbott and Hollenberg (1976); Kogame (1998).

Figure legends:

Figure 1: Maximum Likelihood tree based on ITS1. Both ML and BI trees were congruent, therefore support values are presented as maximum likelihood bootstrap (n=1000) and Bayesian posterior probability, respectively, and – indicates <50% of support. Triangles size proportional to the number of different sequences. –Ln likelihood = 5595,21408.

Figure 2: Maximum Parsimony consensus tree based on ITS1. Numbers at the nodes indicate boostrap values based on 1000 replicates.

Figure 3: Maximum Likelihood tree based on partial *rbc*S-spacer partial *rbc*L. Both ML and BI trees were congruent, therefore support values are presented as maximum likelihood bootstrap (n=1000) and Bayesian posterior probability, respectively, and – indicates <50% of support. Triangles size proportional to the number of different sequences. –Ln likelihood = 2642,67038.

Figure 4: Maximum Parsimony consensus tree based on *rbc*S-spacer partial *rbc*L. Numbers at the nodes indicate boostrap values based on 1000 replicates.

Figure 5: Maximum Likelihood tree based on COX3. Both ML and BI trees were congruent, therefore support values are presented as maximum likelihood bootstrap (n=1000) and Bayesian posterior probability, respectively, and – indicates <50% of support. Triangles size proportional to the number of different sequences. –Ln likelihood = 5419,86073.

Figure 6: Maximum Parsimony consensus tree based on COX3. Numbers at the nodes indicate boostrap values based on 1000 replicates.

Figure 7: Detail of internal morphology of erect thallus of (a) *E. binghamiae*; (b) *P. fascia*; (c) and (d) mix morphological characters of both species. Thin arrow indicates medullary cells and thick arrow indicates medullary filaments.

Figure 8: Non-metric multidimensional scaling (nMDS) plot of morphometric characters of *P. fascia* and *E. binghamiae* along the Chilean coast.

Figure 9: Non-metric multidimensional scaling (nMDS) plot of morphometric characters for (a) *P. fascia* and (b) *E. binghamiae* between localities.

Figure 10: External morphology of erect thallus of *Scytosiphon* species. (a) thin; (b) constrictions; (c) flat and (d) cylindrical. Scale bar = 1 cm.

Figure 11: Non-metric multidimensional scaling (nMDS) plot of morphological characters of *Scytosiphon* species.

Figure 12: Non-metric multidimensional scaling (nMDS) plot of morphological species of *Scytosiphon* genus.

Figure 13: Diagram illustrating the possible combinations between two morphological characters that permit the differentiation of groups of species within de genus *Scytosiphon*.

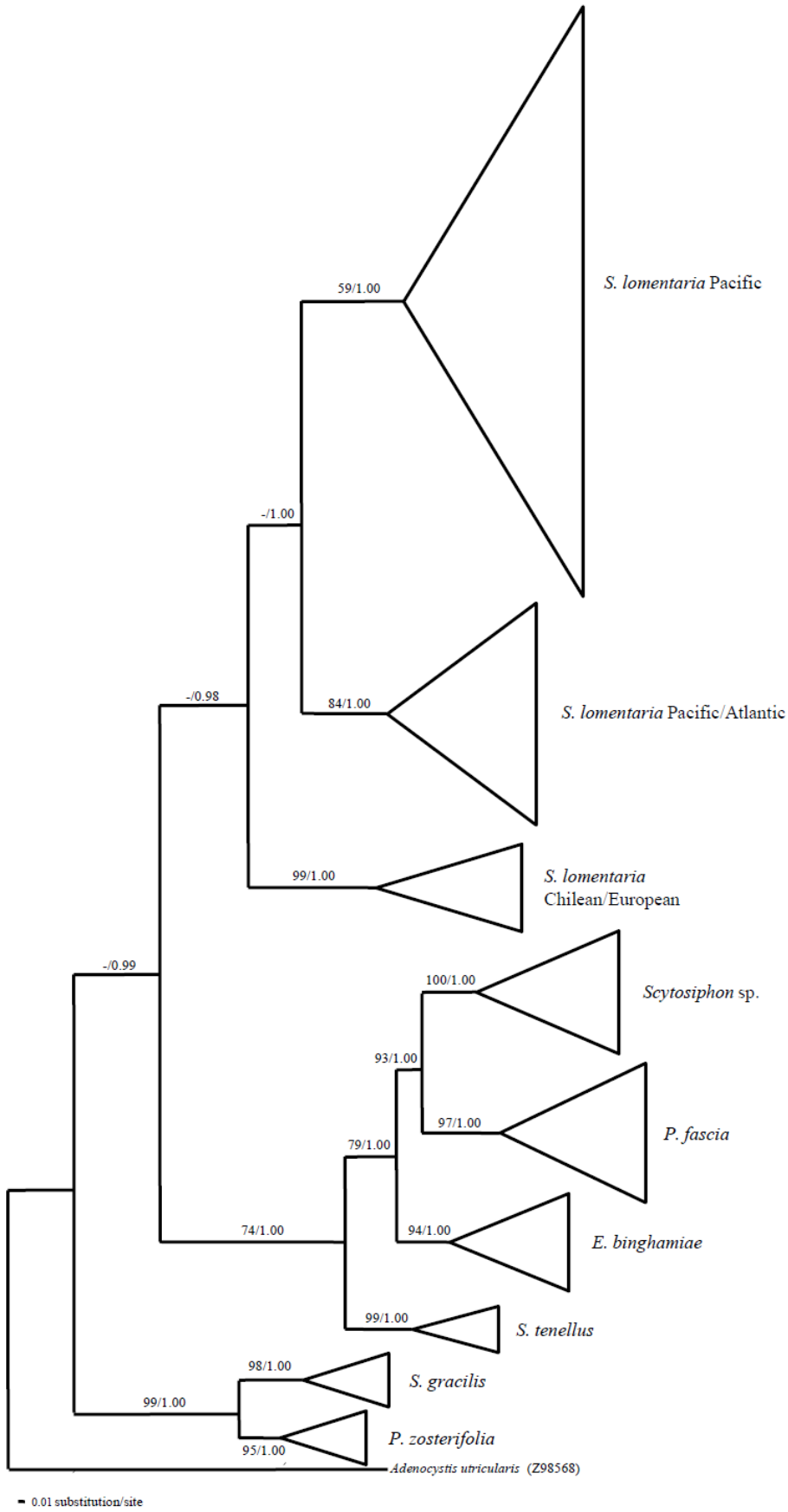
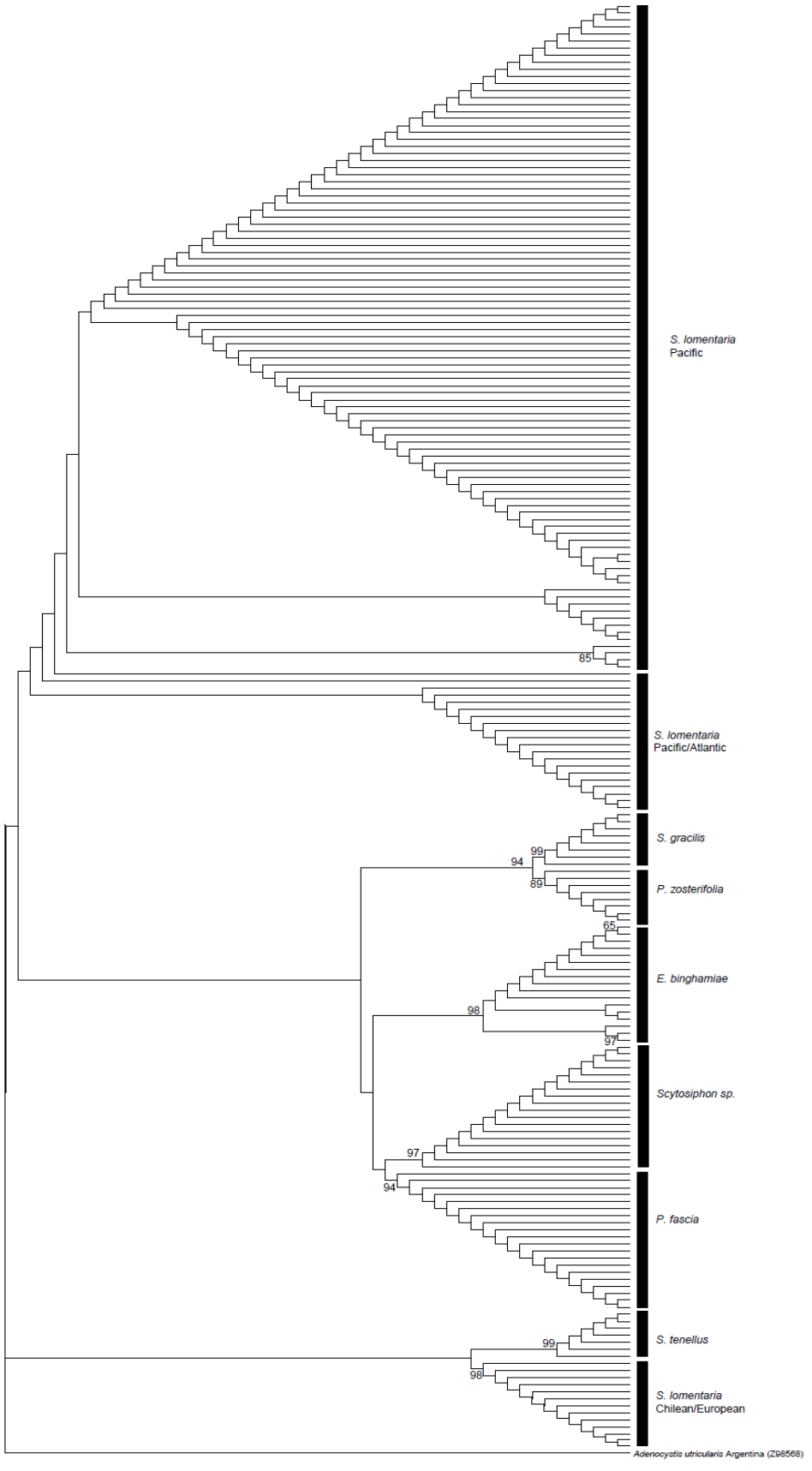

S. lomentaria Pacific
59/1.00
-/1.00
S. lomentaria Pacific/Atlantic
84/1.00
-/0.98
S. lomentaria
Chilean/European
99/1.00
-/0.99
Scytosiphon sp.
100/1.00
93/1.00
P. fascia
97/1.00
79/1.00
E. binghamiae
94/1.00
74/1.00
S. tenellus
99/1.00
S. gracilis
98/1.00
99/1.00
P. zosterifolia
95/1.00
Adenocystis utricularis (Z98568)
0.01 substitution/site

Figure 1.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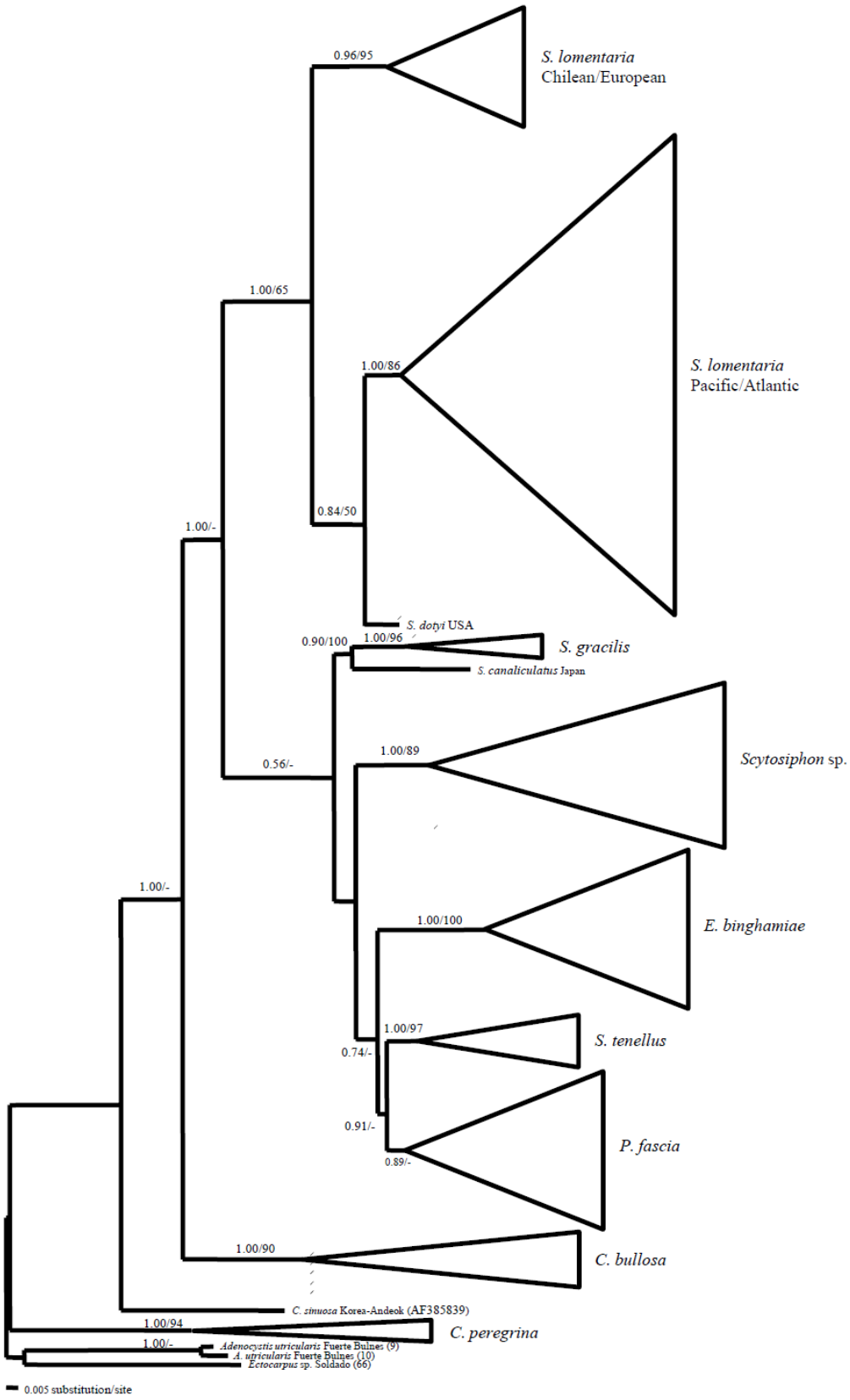
Pacific
85
S. lomentaria
Pacific/Atlantic
S. gracilis
99
94
89
P. zosterifolia
65
E. binghamiae
98
97
Scytosiphon sp.
97
94
P. fascia
S. tenellus
99
98
S. lomentaria
Chilean/European
Adenocystis utricularis Argentina (Z98568)

Figure 2


0.96/95
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Chilean/European
1.00/65
1.00/86
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Pacific/Atlantic
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1.00/-
S. dotyi USA
0.90/100
1.00/96
S. gracilis
S. canaliculatus Japan
1.00/89
Scytosiphon sp.
0.56/-
1.00/-
1.00/100
E. binghamiae
1.00/97
S. tenellus
0.74/-
0.91/-
P. fascia
0.89/-
1.00/90
C. bullosa
C. sinuosa Korea-Andeok (AF385839)
1.00/94
C. peregrina
1.00/-
Adenocystis utricularis Fuerte Bulnes (9)
A. utricularis Fuerte Bulnes (10)
Ectocarpus sp. Soldado (66)
0.005 substitution/site

Figure 3.

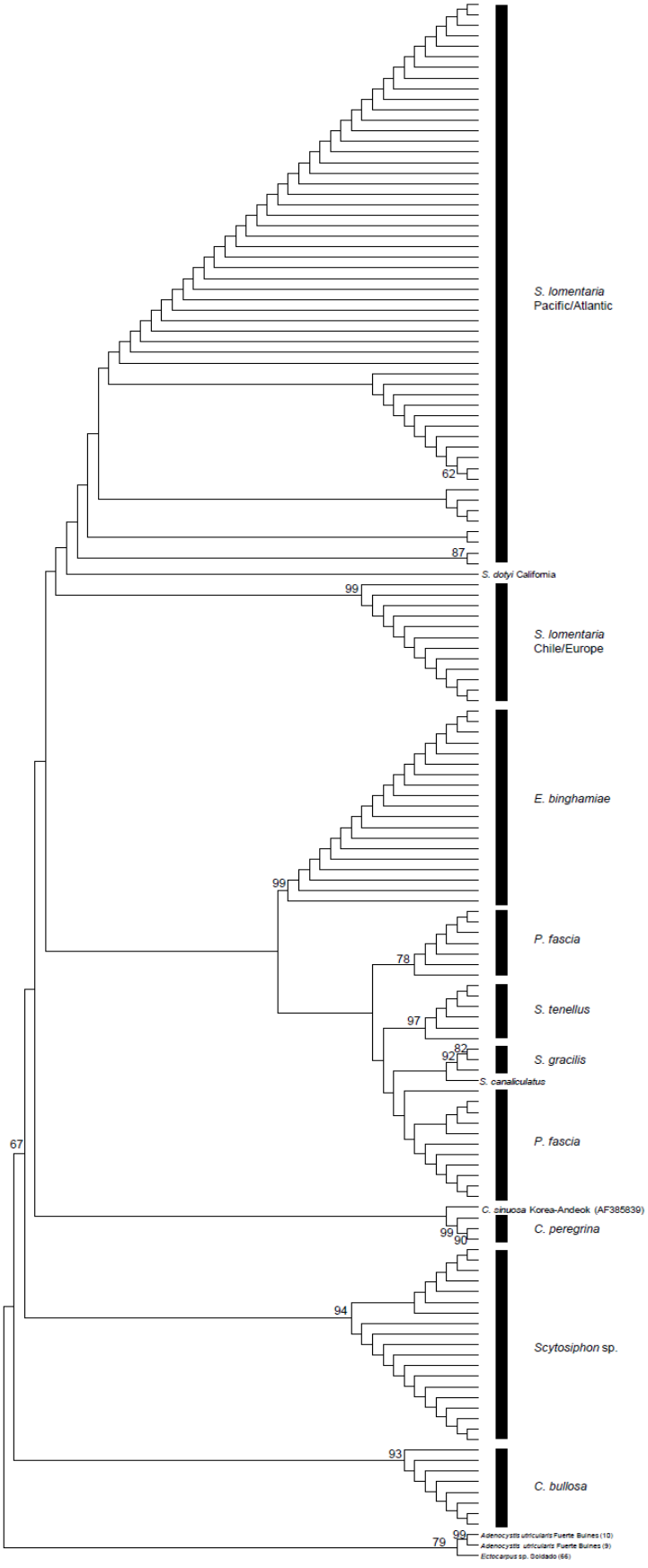
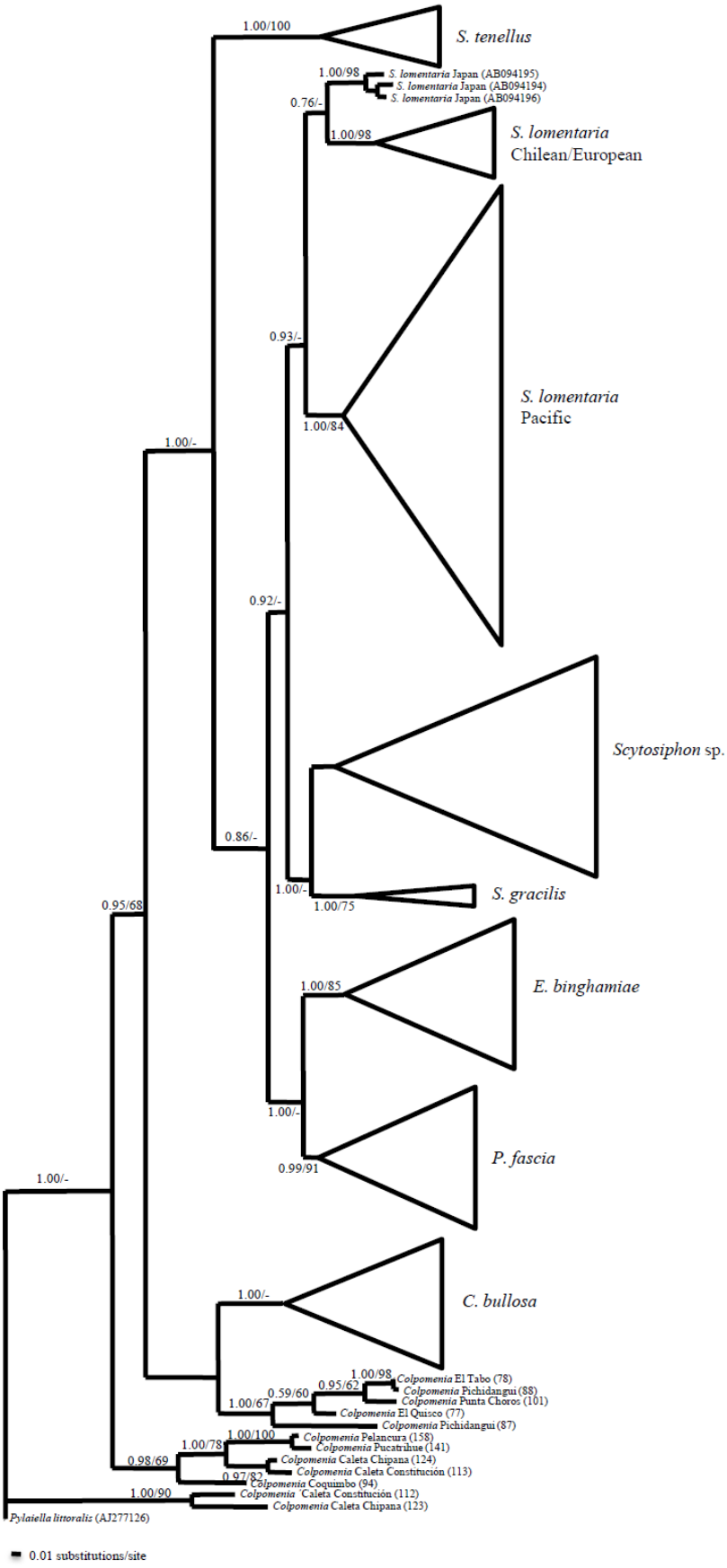

S. lomentaria
Pacific/Atlantic
62
87
S. dotyi California
99
S. lomentaria
Chile/Europe
E. binghamiae
99
P. fascia
78
S. tenellus
97
82
92
S. gracilis
S. canaliculatus
P. fascia
67
C. sinuosa Korea-Andeok (AF385839)
99
90
C. peregrina
94
Scytosiphon sp.
93
C. bullosa
99
79
Adenocystis utricularis Fuerte Bulnes (10)
Adenocystis utricularis Fuerte Bulnes (9)
Ectocarpus sp. Golfado (66)

Figure 4


1.00/100
S. tenellus
1.00/98
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S. lomentaria Japan (AB094194)
S. lomentaria Japan (AB094196)
0.76/-
1.00/98
S. lomentaria
Chilean/European
0.93/-
1.00/84
S. lomentaria
Pacific
1.00/-
0.92/-
Scytosiphon sp.
0.86/-
1.00/-
1.00/75
S. gracilis
0.95/68
1.00/85
E. binghamiae
1.00/-
0.99/91
P. fascia
1.00/-
C. bullosa
1.00/-
1.00/98
Colpomenia El Tabo (78)
0.95/62
Colpomenia Pichidangui (88)
Colpomenia Punta Choros (101)
0.59/60
1.00/67
Colpomenia El Quisco (77)
Colpomenia Pichidangui (87)
1.00/100
Colpomenia Pelancura (158)
1.00/78
Colpomenia Pucatrihue (141)
Colpomenia Caleta Chipana (124)
0.98/69
Colpomenia Caleta Constitución (113)
0.97/82
Colpomenia Coquimbo (94)
1.00/90
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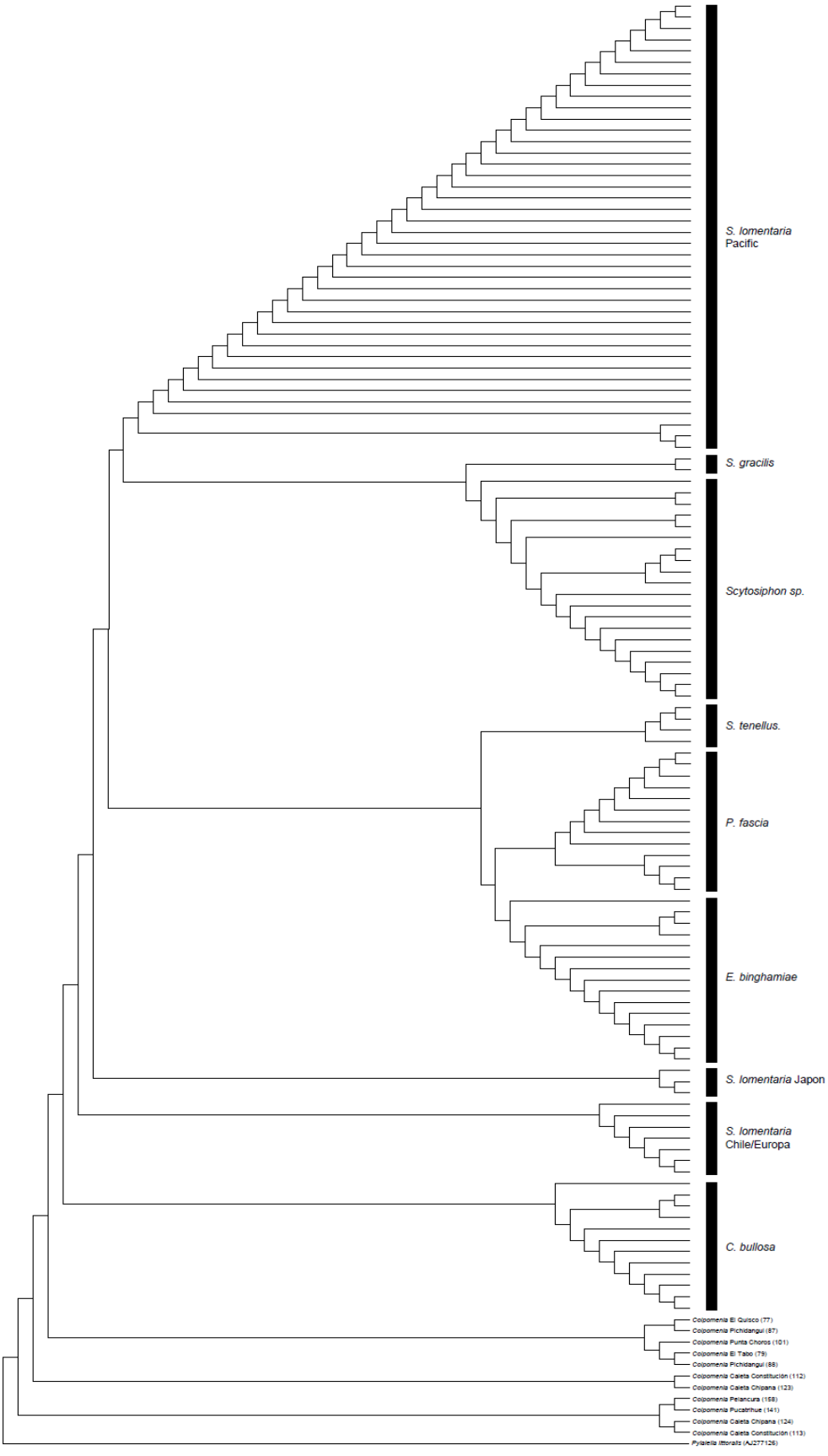
Colpomenia Caleta Chipana (123)
Pylaiella littoralis (AJ277126)
0.01 substitutions/site

Figure 5.


S. lomentaria
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S. gracilis
Scytosiphon sp.
S. tenellus.
P. fascia
E. binghamiae
S. lomentaria Japon
S. lomentaria
Chile/Europa
C. bullosa
Colpomenia El Quisco (77)
Colpomenia Pichidangui (87)
Colpomenia Punta Choros (101)
Colpomenia El Tabo (79)
Colpomenia Pichidangui (88)
Colpomenia Caleta Constitución (112)
Colpomenia Caleta Chipana (123)
Colpomenia Pelancura (158)
Colpomenia Pucatrihue (141)
Colpomenia Caleta Chipana (124)
Colpomenia Caleta Constitución (113)
Pylaiella littoralis (AJ277126)

Figure 6

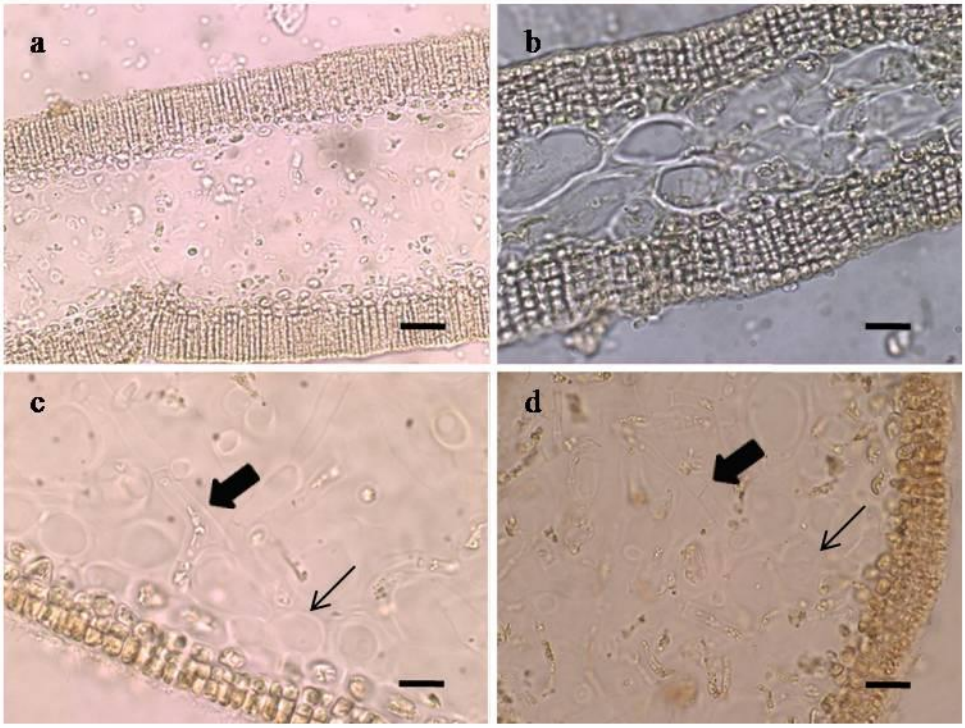


Figure 7.

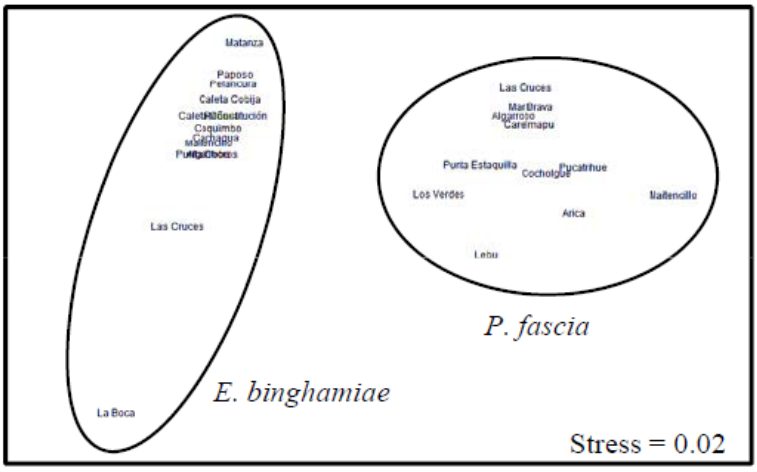

Matanza
Paposo
Pelancura
Caleta Cobija
Caquimbo
Las Cruces
La Boca
E. binghamiae
Las Cruces
MarBrava
Algarrobo
Carelmapu
Punta Estaquilla
Cochoglue
Pucatrihue
Los Verdes
Mallenciillo
Arica
Lebu
P. fascia
Stress = 0.02

Figure 8.

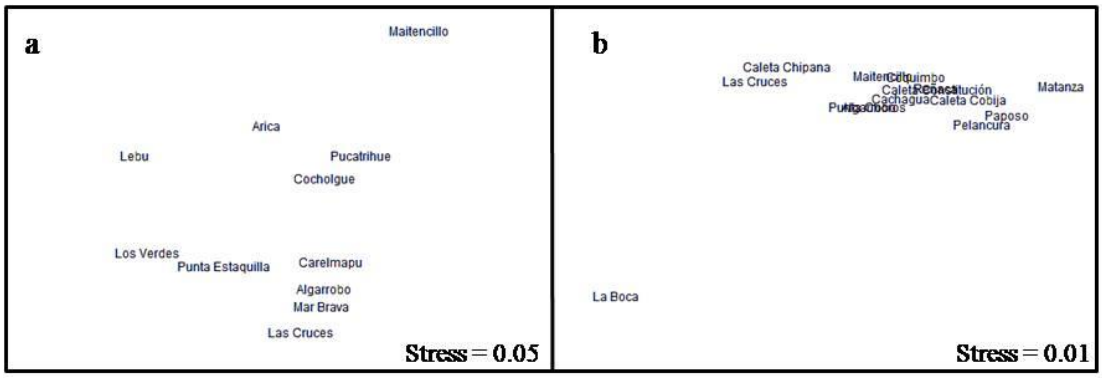

a
Maitencillo
Arica
Lebu
Pucatrihue
Cocholgue
Los Verdes
Punta Estaquilla
Carelmapu
Algarrobo
Mar Brava
Las Cruces
Stress = 0.05
b
Caleta Chipana
Las Cruces
Matanza
Caleta Cobija
Paposo
Pelancura
La Boca
Stress = 0.01

Figure 9.

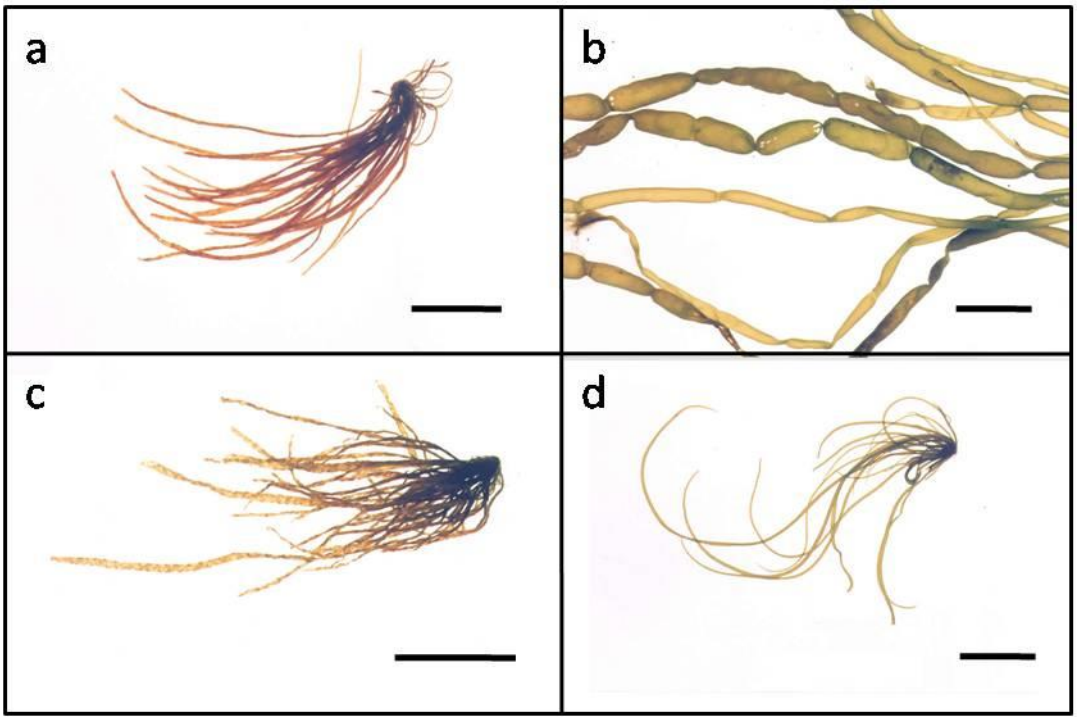


Figure 10.

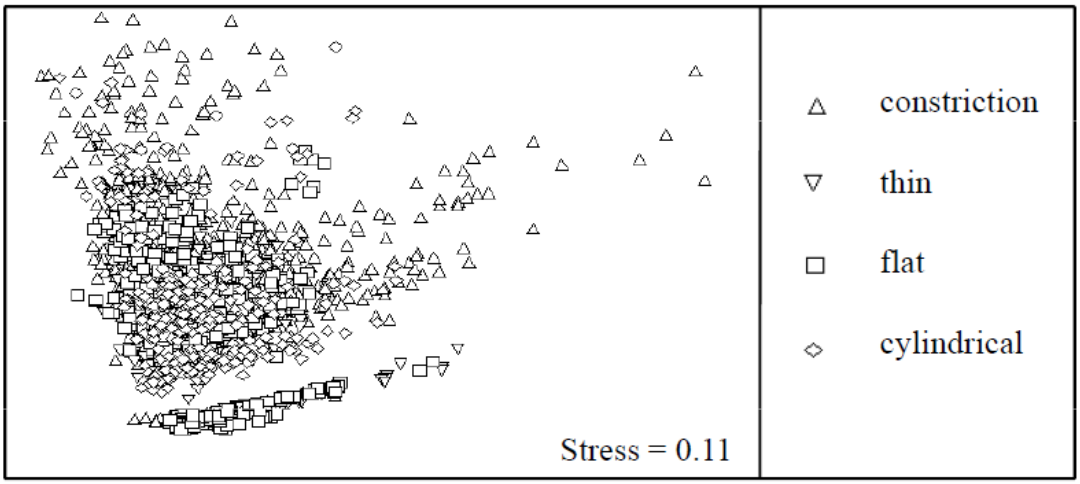


Figure 11.

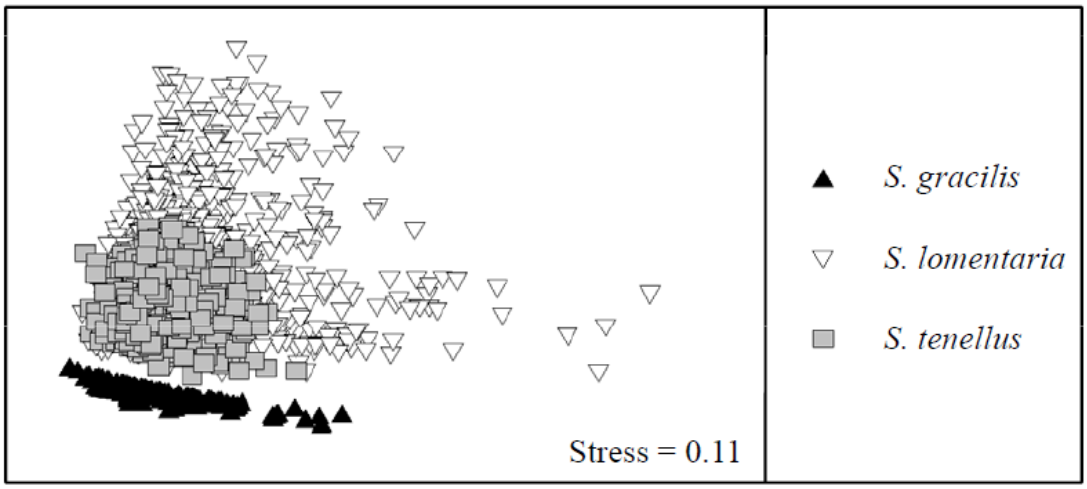


Figure 12.

		Plurilocular sporangia type	
		Loose	Coherent
Ascocysts	Absent		*S. complanatus* *S. crispus* *S. dotyi* *S. gracilis*
	Present	*S. canaliculatus* *S. lomentaria*	*Scytosiphon* sp. *S. tenellus*

Figure 13.

Appendix figure legends:

Figure A1: Maximum likelihood tree based on ITS1. Both ML and BI trees were congruent, therefore support values are presented as maximum likelihood bootstrap (n=1000) and Bayesian posterior probability, respectively, and – indicates <50% of support. –Ln likelihood = 5595,21408.

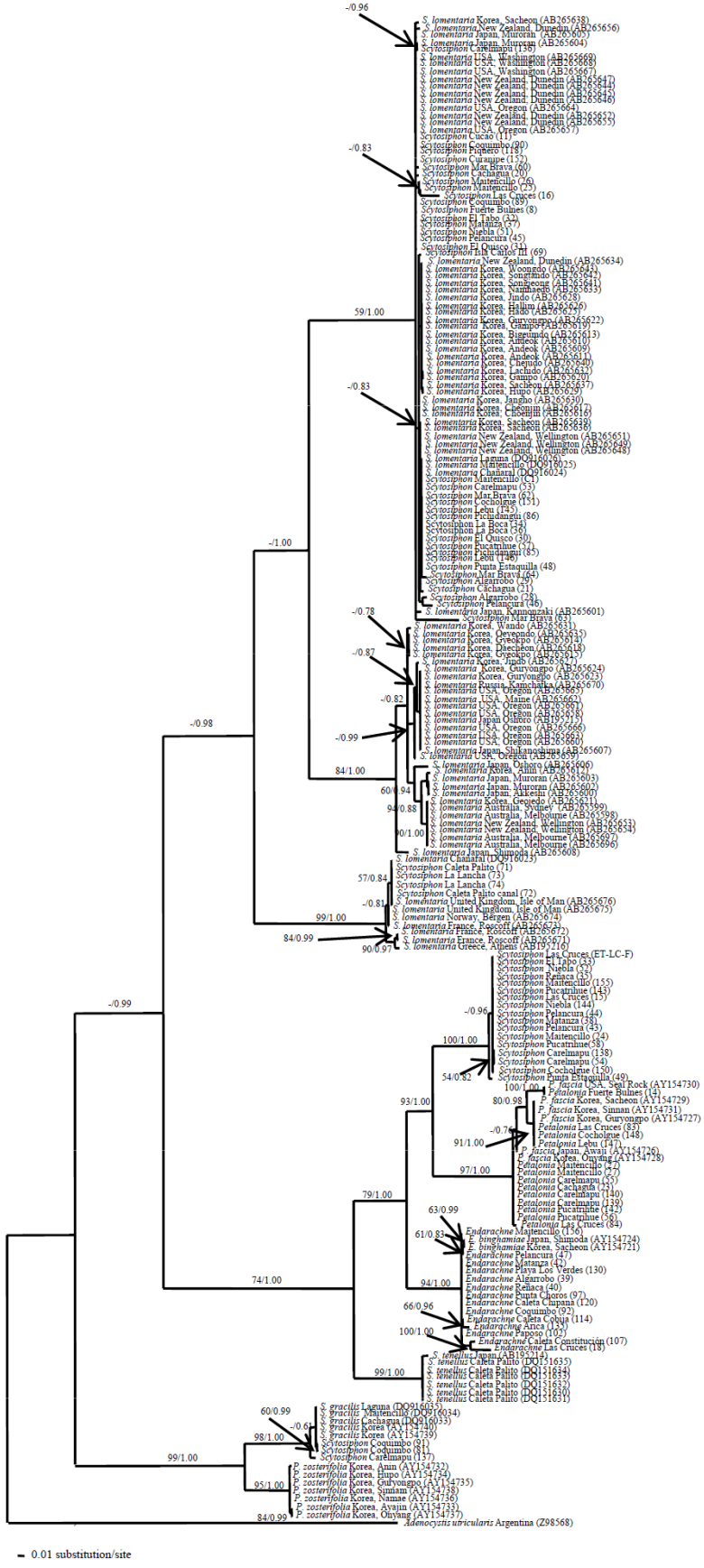
Figure A2: Maximum Parsimony consensus tree based on ITS1. Numbers at the nodes indicate boostrap values based on 1000 replicates.

Figure A3: Maximum likelihood tree based on partial *rbc*S-spacer partial *rbc*L. Both ML and BI trees were congruent, therefore support values are presented as maximum likelihood bootstrap (n=1000) and Bayesian posterior probability, respectively, and – indicates <50% of support. –Ln likelihhod = 2642,67038.

A4: Maximum Parsimony consensus tree based on partial *rbc*S-spacer partial *rbc*L. Numbers at the nodes indicate boostrap values based on 1000 replicates.

Figure A5: Maximum likelihood tree based on COX3 sequences. Both ML and BI trees were congruent, therefore support values are presented as maximum likelihood bootstrap (n=1000) and Bayesian posterior probability, respectively, and – indicates <50% of support. –Ln likelihood = 5419,86073.

Figure A6: Maximum Parsimony consensus tree based on COX3. Numbers at the nodes indicate boostrap values based on 1000 replicates.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S. lomentaria Japan, Muroran (AB265604)
Scytosiphon Carelmapu (138)
S. lomentaria USA, Washington (AB265669)
S. lomentaria USA, Washington (AB265668)
S. lomentaria USA, Washington (AB265667)
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S. lomentaria New Zealand, Dunedin (AB265644)
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S. lomentaria New Zealand, Dunedin (AB265655)
S. lomentaria USA, Oregon (AB265657)
Scytosiphon Cucao (11)
Scytosiphon Coquimbo (90)
Scytosiphon Piquero (118)
Scytosiphon Curaumpe (152)
Scytosiphon Mar Brava (60)
Scytosiphon Cachagua (20)
Scytosiphon Matencillo (26)
Scytosiphon Matencillo (25)
Scytosiphon Las Cruces (16)
Scytosiphon Coquimbo (89)
Scytosiphon Fuerte Bulnes (8)
Scytosiphon El Tabo (32)
Scytosiphon Matanza (37)
Scytosiphon Niebla (31)
Scytosiphon Pelancura (45)
Scytosiphon El Quisco (31)
Scytosiphon Isla Cailos III (69)
S. lomentaria New Zealand, Dunedin (AB265634)
S. lomentaria Korea, Woongsi (AB265642)
S. lomentaria Korea, Songjiando (AB265642)
S. lomentaria Korea, Songjeoug (AB265641)
S. lomentaria Korea, Namhaedo (AB265633)
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S. lomentaria Korea, Heilim (AB265626)
S. lomentaria Korea, Hado (AB265627)
S. lomentaria Korea, Guryongpo (AB265622)
S. lomentaria Korea, Ganpo (AB265619)
S. lomentaria Korea, Bigeumdo (AB265613)
S. lomentaria Korea, Aideok (AB265610)
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S. lomentaria Korea, Sacheon (AB265637)
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S. lomentaria Matencillo (DQ918025)
S. lomentaria Chañaral (DQ916024)
Scytosiphon Matencillo (51)
Scytosiphon Carelmapu (53)
Scytosiphon Mar Brava (62)
Scytosiphon Cocholgue (151)
Scytosiphon Lebu (145)
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Scytosiphon La Boca (34)
Scytosiphon La Boca (36)
Scytosiphon El Quisco (30)
Scytosiphon Pucatrihue (32)
Scytosiphon Pichidangui (85)
Scytosiphon Lebu (146)
Scytosiphon Punta Estaquilla (48)
Scytosiphon Mar Brava (64)
Scytosiphon Algarrobo (29)
Scytosiphon Cachagua (31)
Scytosiphon Algarrobo (28)
Scytosiphon Pelancura (46)
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Scytosiphon Mar Brava (63)
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S. lomentaria Korea, Geoeundo (AB265635)
S. lomentaria Korea, Gyeokpo (AB265614)
S. lomentaria Korea, Daecheon (AB265618)
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S. lomentaria Korea, Jupib (AB265621)
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S. lomentaria Russia, Kamchatka (AB265670)
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Scytosiphon La Lancha (74)
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Scytosiphon Niebla (32)
Scytosiphon Reñaca (35)
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Scytosiphon Las Cruces (15)
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Scytosiphon Matanza (38)
Scytosiphon Pelancura (42)
Scytosiphon Maitencillo (14)
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Scytosiphon Carelmapu (138)
Scytosiphon Carelmapu (54)
Scytosiphon Cocholgue (150)
Scytosiphon Punta Estaquilla (49)
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P. fascia Korea, Sinnan (AY154731)
P. fascia Korea, Guryongpo (AY154727)
Petalonia Las Cruces (83)
Petalonia Cocholgue (148)
Petalonia Lebu (147)
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P. fascia Korea, Ongjing (AY154728)
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0.01 substitution/site

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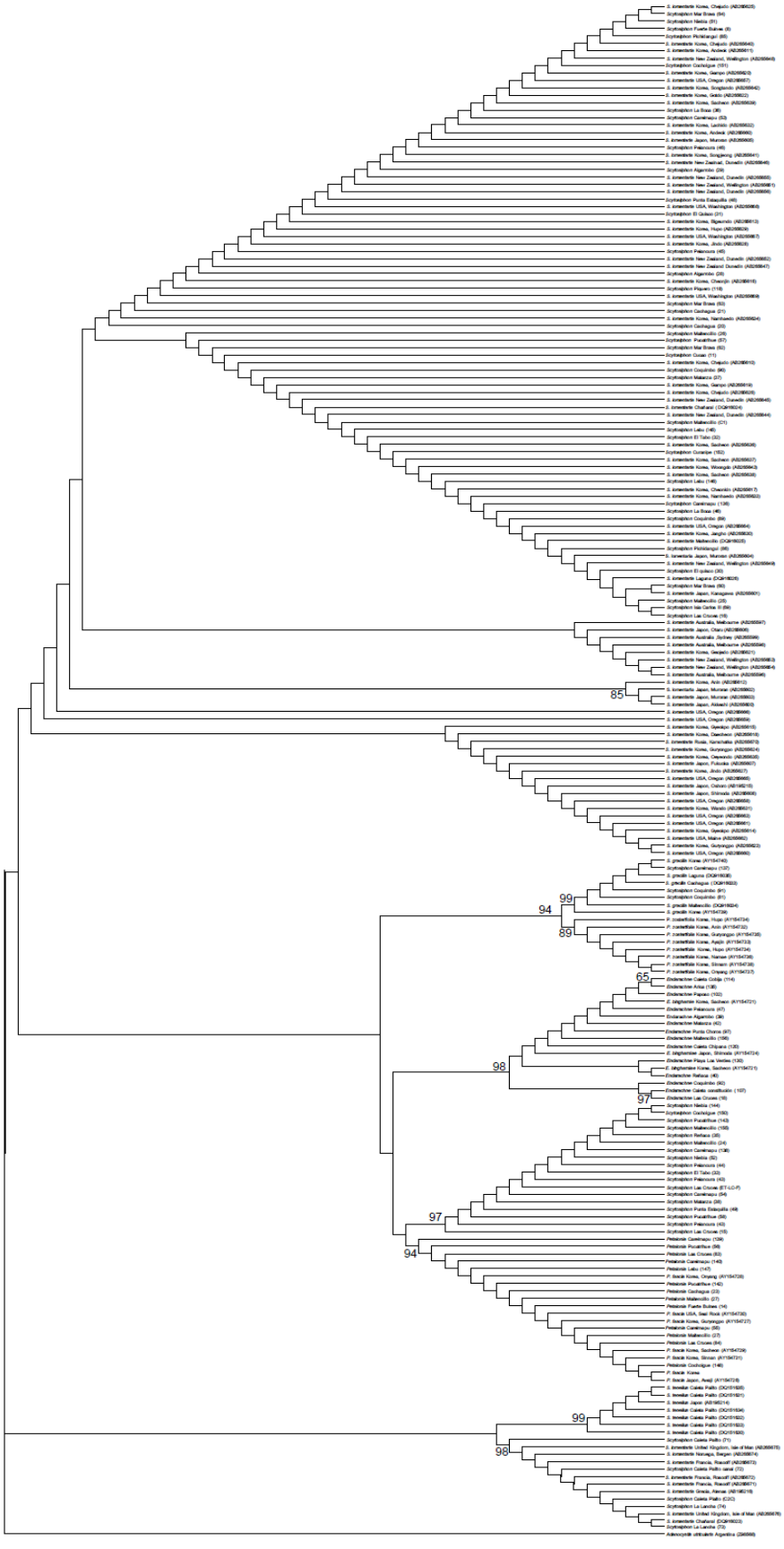

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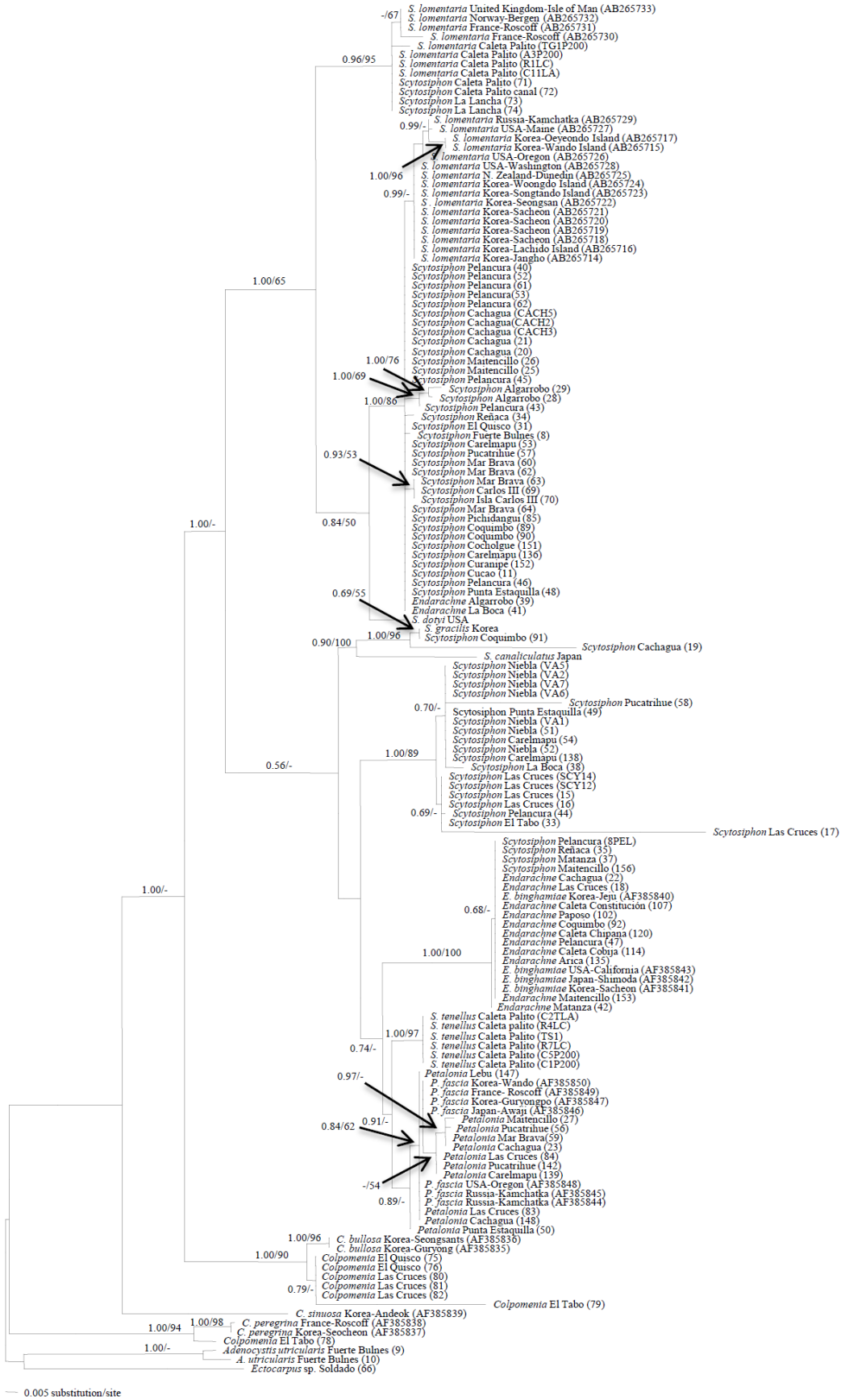
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Scytosiphon La Lancha (74)
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Scytosiphon Cachagua(CACH2)
Scytosiphon Cachagua (CACH3)
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Scytosiphon Mar Brava (62)
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Scytosiphon Isla Carlos III (70)
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S. gracilis Korea
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Scytosiphon Cachagua (19)
S. canaliculatus Japan
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Scytosiphon Niebla (VA2)
Scytosiphon Niebla (VA7)
Scytosiphon Niebla (VA6)
Scytosiphon Pucatrihue (58)
Scytosiphon Punta Estaquilla (49)
Scytosiphon Niebla (VA1)
Scytosiphon Niebla (51)
Scytosiphon Carelmapu (54)
Scytosiphon Niebla (52)
Scytosiphon Carelmapu (138)
Scytosiphon La Boca (38)
Scytosiphon Las Cruces (SCY14)
Scytosiphon Las Cruces (SCY12)
Scytosiphon Las Cruces (15)
Scytosiphon Las Cruces (16)
Scytosiphon Pelancura (44)
Scytosiphon El Tabo (33)
Scytosiphon Las Cruces (17)
Scytosiphon Pelancura (8PEL)
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Endarachne Coquimbo (92)
Endarachne Caleta Chipana (120)
Endarachne Pelancura (47)
Endarachne Caleta Cobija (114)
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E. binghamiae Korea-Sacheon (AF385841)
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Endarachne Matanza (42)
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C. bullosa Korea-Guryong (AF385835)
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Colpomenia El Quisco (76)
Colpomenia Las Cruces (80)
Colpomenia Las Cruces (81)
Colpomenia Las Cruces (82)
Colpomenia El Tabo (79)
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C. peregrina France-Roscoff (AF385838)
C. peregrina Korea-Seocheon (AF385837)
Colpomenia El Tabo (78)
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0.005 substitution/site

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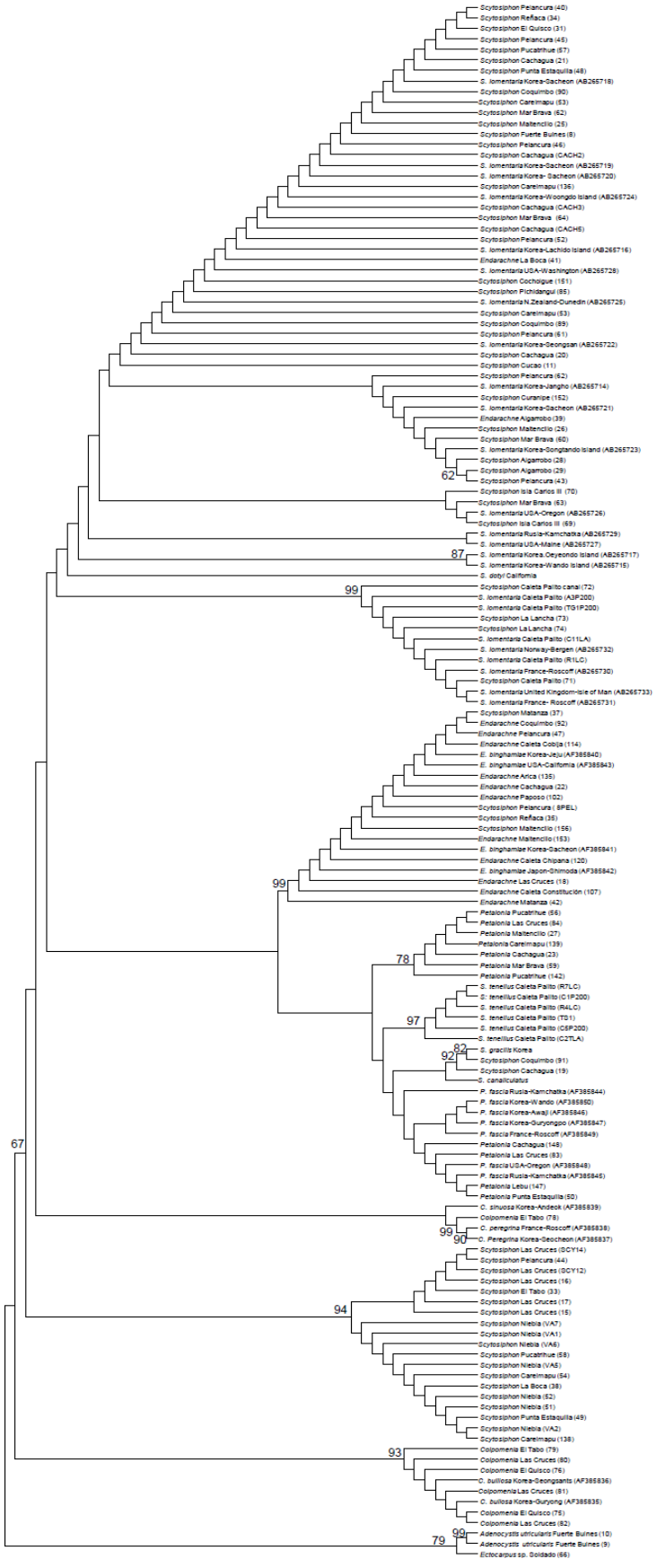
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Scytosiphon Pelancura (46)
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Scytosiphon Coquimbo (89)
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Scytosiphon Cucao (11)
Scytosiphon Pelancura (62)
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Colpomenia El Quisco (76)
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Adenocystis utricularis Fuerte Bulnes (9)
Ectocarpus sp. Soldado (66)
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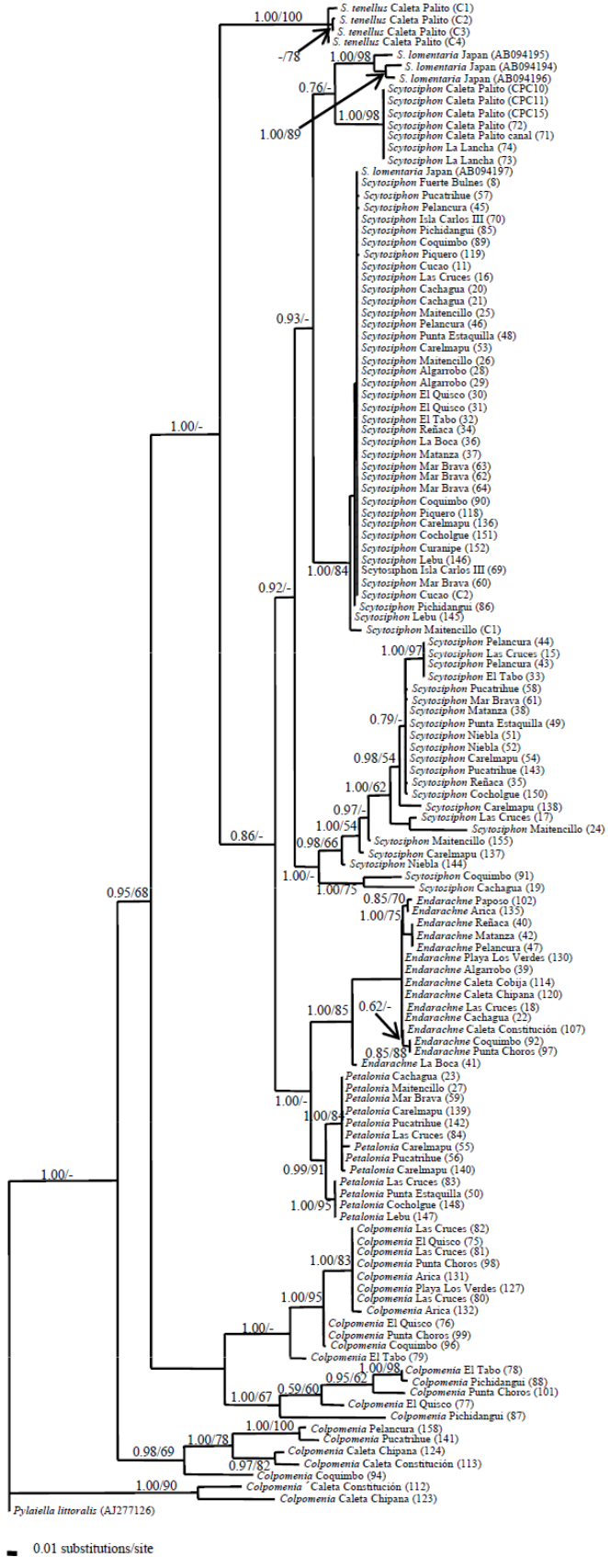
99
79

Figure A4.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Scytosiphon Cucao (11)
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0.01 substitutions/site

Figure A5.

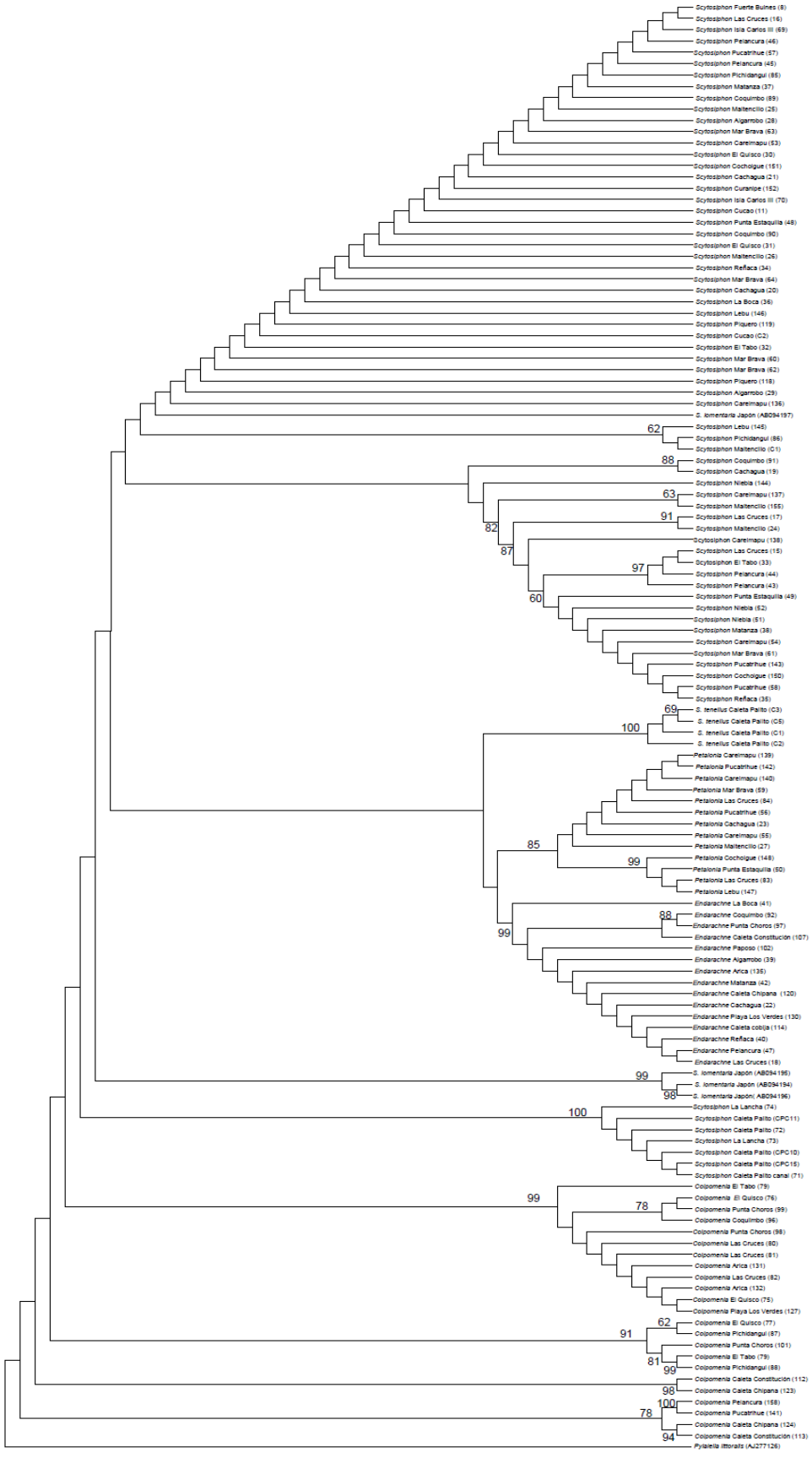
Scytosiphon Fuerte Bulnes (8)
Scytosiphon Las Cruces (16)
Scytosiphon Isla Carlos III (69)
Scytosiphon Pelancura (46)
Scytosiphon Pucatrihue (57)
Scytosiphon Pelancura (45)
Scytosiphon Pichidangui (85)
Scytosiphon Matanza (37)
Scytosiphon Coquimbo (89)
Scytosiphon Maitencillo (25)
Scytosiphon Algarrobo (28)
Scytosiphon Mar Brava (63)
Scytosiphon Careimapu (53)
Scytosiphon El Quisco (30)
Scytosiphon Cocholgue (151)
Scytosiphon Cachagua (21)
Scytosiphon Curanipe (152)
Scytosiphon Isla Carlos III (70)
Scytosiphon Cucao (11)
Scytosiphon Punta Estaquilla (48)
Scytosiphon Coquimbo (90)
Scytosiphon El Quisco (31)
Scytosiphon Maitencillo (26)
Scytosiphon Reñaca (34)
Scytosiphon Mar Brava (64)
Scytosiphon Cachagua (20)
Scytosiphon La Boca (36)
Scytosiphon Lebu (146)
Scytosiphon Piquero (119)
Scytosiphon Cucao (C2)
Scytosiphon El Tabo (32)
Scytosiphon Mar Brava (60)
Scytosiphon Mar Brava (62)
Scytosiphon Piquero (118)
Scytosiphon Algarrobo (29)
Scytosiphon Careimapu (136)
S. lomentaria Japón (AB094197)
62
Scytosiphon Lebu (145)
Scytosiphon Pichidangui (86)
Scytosiphon Maitencillo (C1)
88
Scytosiphon Coquimbo (91)
Scytosiphon Cachagua (19)
Scytosiphon Niebla (144)
63
Scytosiphon Careimapu (137)
Scytosiphon Maitencillo (155)
91
Scytosiphon Las Cruces (17)
82
Scytosiphon Maitencillo (24)
Scytosiphon Careimapu (138)
87
Scytosiphon Las Cruces (15)
Scytosiphon El Tabo (33)
97
Scytosiphon Pelancura (44)
Scytosiphon Pelancura (43)
60
Scytosiphon Punta Estaquilla (49)
Scytosiphon Niebla (52)
Scytosiphon Niebla (51)
Scytosiphon Matanza (38)
Scytosiphon Careimapu (54)
Scytosiphon Mar Brava (61)
Scytosiphon Pucatrihue (143)
Scytosiphon Cocholgue (150)
Scytosiphon Pucatrihue (58)
Scytosiphon Reñaca (35)
69
S. tenellus Caleta Palito (C3)
S. tenellus Caleta Palito (C5)
100
S. tenellus Caleta Palito (C1)
S. tenellus Caleta Palito (C2)
Petalonia Careimapu (139)
Petalonia Pucatrihue (142)
Petalonia Careimapu (140)
Petalonia Mar Brava (59)
Petalonia Las Cruces (84)
Petalonia Pucatrihue (56)
Petalonia Cachagua (23)
Petalonia Careimapu (55)
85
Petalonia Maitencillo (27)
99
Petalonia Cocholgue (148)
Petalonia Punta Estaquilla (60)
Petalonia Las Cruces (83)
Petalonia Lebu (147)
Endarachne La Boca (41)
88
Endarachne Coquimbo (92)
99
Endarachne Punta Choros (97)
Endarachne Caleta Constitución (107)
Endarachne Paposo (102)
Endarachne Algarrobo (39)
Endarachne Arica (135)
Endarachne Matanza (42)
Endarachne Caleta Chipana (120)
Endarachne Cachagua (22)
Endarachne Playa Los Verdes (130)
Endarachne Caleta cobija (114)
Endarachne Reñaca (40)
Endarachne Pelancura (47)
Endarachne Las Cruces (18)
99
S. lomentaria Japón (AB094196)
S. lomentaria Japón (AB094194)
98
S. lomentaria Japón (AB094196)
Scytosiphon La Lancha (74)
100
Scytosiphon Caleta Palito (CPC11)
Scytosiphon Caleta Palito (72)
Scytosiphon La Lancha (73)
Scytosiphon Caleta Palito (CPC10)
Scytosiphon Caleta Palito (CPC15)
Scytosiphon Caleta Palito canal (71)
Colpomenia El Tabo (79)
99
Colpomenia El Quisco (76)
78
Colpomenia Punta Choros (99)
Colpomenia Coquimbo (96)
Colpomenia Punta Choros (98)
Colpomenia Las Cruces (80)
Colpomenia Las Cruces (81)
Colpomenia Arica (131)
Colpomenia Las Cruces (82)
Colpomenia Arica (132)
Colpomenia El Quisco (75)
Colpomenia Playa Los Verdes (127)
62
Colpomenia El Quisco (77)
91
Colpomenia Pichidangui (87)
Colpomenia Punta Choros (101)
81
Colpomenia El Tabo (79)
99
Colpomenia Pichidangui (88)
Colpomenia Caleta Constitución (112)
98
Colpomenia Caleta Chipana (123)
100
Colpomenia Pelancura (158)
78
Colpomenia Pucatrihue (141)
Colpomenia Caleta Chipana (124)
94
Colpomenia Caleta Constitución (113)
Pylaiella littoralis (AJ277126)

Figure A6.

CHAPTER 2

Multiple origins promote multiple lineages in *Scytosiphon* (Scytosiphonaceae, Ectocarpales) along the Southeastern Pacific Coast

ABSTRACT

In the present study, the genetic diversity of species belonging to the genus *Scytosiphon* present in the Southeastern Pacific coast was analyzed using the mitochondrial COX3 to characterize the geographical distribution and genetic diversity of the different taxonomic entities of the genus and to infer the historical processes that may have determined such lineage distribution. Through sequence-based species delimitation and phylogenetic reconstructions we identified four lineages within the genus along the Southeastern Pacific Coast. *S. lomentaria* and *Scytosiphon* sp. are co-distributed along central and southern Chile, the latter with some discontinuities, whereas *S. gracilis* and *S. lomentaria* Chilean/European are restricted to one or two localities. However, these latter species present higher genetic variability than the widely distributed ones. The results also suggest that the four species present different spatio-temporal origins based on their current geographic distribution and genetic diversity. It is suggested that the species with restricted distribution recently colonized the region, following human-induced introduction, while *S. lomentaria* would have come through an ancestral event of colonization. Finally, *Scytosiphon* sp. would have diverged from *S. gracilis* through an event of vicariance.

RESUMEN

En el presente estudio, la diversidad genética de las especies que pertenecen al género *Scytosiphon* presentes en la costa del Pacifico sureste fue analizada a través del marcador mitocondrial COX3 para caracterizar la distribución geográfica y diversidad genética de las diferentes entidades taxonómicas del género y para inferir los procesos históricos que determinaron la distribución de aquellos linajes. A través de un método de delimitación de especies basado en secuencias y reconstrucciones filogenéticas identificamos cuatro linajes dentro del género a lo largo de la costa del Pacifico Sureste. *S. lomentaria* y *Scytosiphon* sp. se encuentran co-distribuidos a través del centro y sur de Chile, presentando esta última especie algunas discontinuidades, mientras que *S. gracilis* y *S. lomentaria* Chileno/Europeo se encuentran restringidos a una o dos localidades a lo largo de la región. Sin embargo, las especies con distribución restringida presentan mayor variabilidad genética que las especies ampliamente distribuidas. Los resultados también sugieren que las especies presentan diferentes orígenes espacio temporales basado en la distribución geográfica actual y la diversidad genética que muestran. Se propone que las especies con distribución restringida colonizaron recientemente la región a través de eventos de introducción mediados por el hombre mientras que *S. lomentaria* habría llegado a través de eventos de colonización ancestrales. Finalmente, *Scytosiphon* sp. habría derivado de *S. gracilis* a través de un evento de vicarianza.

1. INTRODUCTION

Global environmental changes and geological events have repeatedly modified the distribution ranges of species over ecological and evolutionary time scales. Species range evolution over time leaves genetic signatures at population and species levels, which can be investigated using phylogeographic analyses (Avise, 2000). The major goal of phylogeography is indeed to understand the principles and process governing the geographic distribution of genealogical lineages, especially those within and among closely related species (Avise et al., 1987). By comparing the evolutionary relationships of genetic lineages with their geographical locations, we may gain a better understanding of which factors have most influenced the distribution of genetic variation (Avise, 2000). Recently, comparative phylogeography, as the use of phylogeographic analyses applied to co-distributed species, have been extensively used to elucidate common historical features of intra- and inter-specific diversification (Bermingham and Moritz 1998; Avise, 2000). Concordant phylogeographical patterns of sympatric species can be driven by common physical process, while, divergent pattern among species can be related to different dispersal ability, species-specific ecological requirement or different historical events. Furthermore, phylogeographic studies of closely related species with diverse levels of sympatry is of particular interest to understand speciation patterns and processes driving the distribution of lineages in each particular species.

Terrestrial species are often restricted to particular continental land masses, whereas marine taxa have generally been regarded as having very large geographical ranges (Andreakis et al., 2007). It is increasingly recognized, however, that although widespread marine species can appear uniform throughout their range, each species

may have diverged into several lineages geographically separated (Andreakis et al., 2007). Since Knowlton`s (1993) seminal review of sibling species in the sea, the recognition of cryptic species has become a dominant theme in phylogegraphy as well as taxonomic research into marine taxa (Jolly et al., 2006; Lee and Foighil, 2004; Schwaninger, 2008; Knowlton, 2000). Cryptic taxa are particularly problematic when anthropogenic invasions are involved, as new introductions may be overlooked and sources are difficult to identify. Invasion of a community by a species that is morphologically similar to a resident member will likely be undetected, as will invasions by more than one member of a sibling species complex (Geller et al., 1997). Lack of diagnostic characters is particularly frequent in marine algae. Here, I studied the *Scytosiphon* genus present in South-eastern Pacific coasts.

For seaweeds, phylogeographic approaches have shown that many apparently widely distributed taxa are composed of biologically and genetically distinct but morphologically cryptic or pseudo-cryptic species (*sensu* Knowlton, 1993). Each of these newly recognized taxa may itself be widely distributed but is generally seen to have a more restricted range than the species complex (e.g. *Bostrychia radicans*/*B. moritziana* (Zuccarello and West, 2003); *Halimeda* section *Halimeda* (Verbruggen et al., 2005a); *Halimeda* section *Rhipsalis* (Verbruggen et al. 2005b); *Boodlea* (Leliaert et al., 2009); *Lessonia nigrescens* (Tellier et al. 2009)). Species of the genus *Scytosiphon* constitute such widely distributed taxa with multiple disjunctions, some of which are believed to be the product of recent introductions. In this study we focus on four (*S. lomentaria*, *S. gracilis*, *Scytosiphon* sp., *S. lomentaria* European/Chilean) of the seven species of the genus distributed along the southeastern Pacific coast. This genus appears as an interesting model because it presents species with contrasting geographical distribution and high genetic variability. On one hand, it present species

with a continuous distribution along the coast of the Southeast Pacific, and on the other hand two species with a highly localized distribution, present in one or two localities. Also, the *S. lomentaria* reported in Chapter 1, is composed of at least two different lineages (cryptic diversity): one corresponds to the worldwide distributed species (*S. lomentaria*) and the second correspond to individuals that present more similarity with the species that inhabits Europe (*S. lomentaria* Chilean/European). Finally, *Scytosiphon sp*. appears as a species whose region of origin would have been the Southeast Pacific coast, since it is not present in other regions of the world (see Chapter 1).

The mitochondrial COX3 gene was used to characterize the geographical distribution and genetic diversity of the different taxonomic entities of the genus and to infer its historical-geographical origin. The mitochondrial marker was selected because of their inherent characteristics (no recombination, maternal inheritance, haploid) and also because it has been shown to provide good resolution at the species level, at least in *S. lomentaria* (Kogame et al., 2005). Given the inherent difficulties of identifying species in this morphologically variable group of algae (Chapter 1), we sampled all the different morphotypes and, through a sequence-based species delimitation method and a phylogenetic reconstruction, we assigned individuals to species.

2. MATERIALS AND METHODS

2.1. Taxa sampling

Due to the high variability of external morphological characters and the lack of diagnostic characters for the different species of the genus, we collected constricted, cylindrical, flat and thin thalli in each of 14 localities, during low tide from rocky platforms at the intertidal zone (Table 1, Fig. 1). At each locality 30 individuals of each morphotype were sampled and immediately placed in individual plastic bags filled with silica crystals for rapid dehydration.

2.2. PCR primers and conditions

Small fragments of dried fronds were placed in tubes with stainless steel beads and ground to fine powder in a Mini Beadbeater (Biospec Products, INC, Bartlesville, OK, USA). The remaining of the sample was deposited in the Colección Flora y Fauna Profesor Patricio Sánchez Reyes (SSUC), Departamento de Ecología, Pontificia Universidad Católica de Chile, Santiago, Chile. Total genomic DNA was extracted using the Ultra clean Soil DNA Kit (MoBio Laboratories, Carlsbad, CA, USA), following the manufacturer`s instructions. For each sample, the mitochondrial COX3 was amplified using primers CAF4A and CAR4A (designed by Kogame et al., 2005). The PCR reactions (14.5μL total volume) contained 1μL of DNA, 10x reaction buffer (Invitrogen, Carlsbad, CA, USA), 50 mM $MgCl_2$ (Invitrogen), 2.5 mM of each dNTP (Fermentas, Burlington, Ontario, Canada), 5 U/μL Taq DNA polymerase (Fermentas), 10x BSA (New England, BioLabs, Ipswich, MA, USA) and 10 μM of each primer. The reaction profile for COX3 was 95°C for 8 min followed by 94°C for 1 min, 55°C for 45 sec, 72°C for 2 min for 35 cycles, and a final extension at 72°C for

7 min. PCR products were visualized with ethidium bromide staining after electrophoresis in a 2% agarose gel.

2.3. SSCP

To investigate the polymorphism of the COX3 marker at the population level I used the screening method of single-strand conformation polymorphism (SSCP) (Orita et al., 1989; Sunnucks et al., 2000). SSCP takes advantages of the fact that single-stranded (denatured) DNA molecules of few hundred bp length often adopt different conformations even when differing by as few as one nucleotide. These distinctive conformations can be detected by electrophoresis of the PCR-amplified molecules through neutral polyacrylamine gels (Avise, 2004).

To perform this technique, 10 µL of PCR product were mixed with 16 µL of denaturing/loading buffer containing 8 µL of 15% ficoll loading buffer (with 0.25% bromophenol blue and 0.25% xylene cyanol), 5 µL of urea 5 M and 19.7 µL TBE 1 x buffer. After denaturing for 15 min at 95ºC, amplification products were separated using 10% polyacrylamide (37.5:1 acrylamide:bisacrylamide) gels run in 0.5 x TBE buffer at 250 V during 17 h at 4ºC on a vertical electrophoresis system (Bio-Rad, Hercules, California, USA). After electrophoresis, SSCP gels were stained for 20 min with 2.5 x Sybr Gold solution and bands were visualized under UV light. To check for accuracy of the SSCP typing, we sequenced every different SSCP profile obtained on each SSCP gel. For this purpose, we purified the PCR products with a QIAquick PCR purification kit (Qiagen, Duesseldorf, Germany) following the manufacturer´s instructions or purified by Macrogen (Seoul, Korea). Sequences were determined using a 3100 Genetic Analyser (Applied Biosystems, CA, USA) or outsourced to Macrogen (Seoul, Korea).

2.4. Phylogenetic analysis

Two datasets were considered: Dataset 1 including 28 sequences obtained in this study and Dataset 2 including the sequences obtained in this study plus previously published sequences (Table 2). Both datasets were aligned using CLUSTALW (Thompson et al., 1994) implemented in BIOEDIT (Hall, 1999) and *Pylaiella littoralis* (GenBank accession number: AJ277126) was used as outgroup. The amount of phylogenetic signal was assessed using the *Iss* statistic, a measure of substitution saturation in molecular phylogenetic datasets. The DAMBE software (Xia and Xie, 2001) was used to calculate *Iss* values and compare them against critical *Iss* values for symmetric and asymmetric topologies (Xia et al., 2003). The substitution saturation test showed that both datasets did not suffer from saturation (dataset 1: $Iss = 0.092 < Iss.c = 0.708$, $p < 0.000$; dataset 2: $Iss = 0.469 < Iss.c = 0.707$, $p < 0.003$). Once saturation was discarded, Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference (BI) were used. MP analysis was carried out using MEGA5 (Tamura et al 2011). ML analyses were performed in PAUP v.4.0b10 (Swofford, 2003) using a heuristic search with random stepwise addition and tree bisection-reconnection branch swapping algorithm to search for the tree that best meets the optimality criterion. Support was estimated using 1,000 bootstrap replicates. To determine the most appropriate model for the data, Likelihood Ratio Test (LRT) from ModelTest version 3.7 (Posada and Crandall, 1998) was used. For BI, the program MrModelTest version 2.3. (Nylander, 2004) was used to determine the optimal model of sequence evolution, using the Akaike Information Criterion (AIC). The phylogeny was constructed using MrBayes version 3.2. (Huelsenbeck and Ronquist, 2000; Ronquist and Huelsenbeck, 2003). Two independent Metropolis-Coupled Markov

Chain Monte Carlo (MCMCMC) analyses were conducted during 4,000,000 generations with four incremental heated chains. Four runs were conducted and each run was sampled every 1,000th generation. The default parameters were used for temperature and swapping. Examination of the –ln likelihood (-ln L) scores indicated that stability was reached in the first 100,000 generations; therefore, to ensure stability, the first 10,000 generations were discarded as the "burn-in" phase and the remaining trees were used to compute the consensus tree.

For Dataset 1, the selected model of evolution was TVM+I+G (base frequencies A: 0.2088 / C: 0.1748 / G: 0.1961 / T: 0.4203; substitution rates A-C: 3.8656 / A-G: 12.3522 / A-T: 3.4709 / C-G: 1.2409 / C-T: 12.3522 / G-T: 1.000; among site rate variation: 0.4744; gamma distribution shape parameter: 0.8162). For the Dataset 2, the selected model of evolution for ML analysis was TrN+G (base frequencies A: 0.2233 / C: 0.1691 / G: 0.1847 / T: 0.4230; substitution rates A-C: 1.0000 / A-G: 2.9499 / A-T: 1.0000 / C-G: 1.0000 / C-T: 5.0994 / G-T: 1.000; gamma distribution shape parameter: 0.2712) and GTR+G (base frequencies A: 0.2034 / C: 0.1695 / G: 0.2052 / T: 0.4220; substitution rates A-C: 1.9142 / A-G: 3.9363 / A-T: 1.9059 / C-G: 0.7697 / C-T: 7.3926 / G-T: 1.000; gamma distribution shape parameter: 0.2807) for BI.

2.5. Phylogeographic analysis

To analyse the genetic composition of the *Scytosiphon* lineages I estimated the number of polymorphic sites, phylogenetically informative sites, average number of nucleotide differences and fixed differences, as well as haplotype and nucleotide diversity, using DNAsp version 5.0 (Rozas et al., 2003). Intra-specific relationships among mtDNA haplotypes for each lineage were inferred from Minimum Spanning

Networks (MSN) using the Median-Joining method available in Network version 4.5.1.0 (www.fluxus-technology.com). Demographic expansions were examined with Tajima´s D, Fu and Li neutrality tests also performed in DNAsp.

3. RESULTS

After screening 576 individuals from 14 localities along the Chilean coast, twenty eight COX3 haplotypes were recovered (GenBank accession numbers from GU252676 to GU252703). These haplotypes were 555 bp long and the alignment presented 131 polymorphic sites (Fig. 2). Four highly supported monophyletic clusters were recovered from the phylogeny : Lineage 1 composed of individuals of two localities in northern Chile (CP and CPC) which corresponds to the European/Chilean *S. lomentaria* of Chapter 1; Lineage 2 that corresponds to *S. gracilis* represented only by individuals from COQ; Lineage 3 that corresponds to *Scytosiphon* sp., inhabiting the coast of central and southern Chile from MAI to CAR (1,030 Km); and Lineage 4 including individuals from COQ to CAR (1,330 Km), which corresponds to world-wide distributed *S. lomentaria* Chilean/Pacific of Chapter 1 (locality abbreviation as in Table 1 and Fig. 3). Also, the high number of fixed differences between lineages supports the clustering described above, i.e. four independent lineages, being *S. gracilis* the most dissimilar species with 66 to 80 polymorphic sites where all its sequences are different from the other species (Table 3). After having clearly identified the different lineages of *Scytosiphon* along the Chilean coast and assigned them to the corresponding species, we used Dataset 2 (published sequences of *Scytosiphon* species along the Chilean coast plus the 28 haplotype sequences) to construct a new phylogeny (Figure 4). The alignment was 518 base-pair long, for 112 taxa. In the phylogeny it is possible to differentiate the already mentioned four lineages and three other species reported for the Chilean coasts appeared included (e.g. *E. binghamiae*, *P. fascia*, *S. tenellus*) (Fig. 4). The relationships between the lineages remains the same as in Figure 3, *S. gracilis* and

Scytosiphon sp. appear consistently together, and *S. lomentaria* Chilean/European as the most external lineage and clearly differentiated from classic *S. lomentaria* (Chilean/Pacific). Therefore, we excluded the possibility that the two clades of *S. lomentaria* were the product of sampling widely divergent individuals of the same species or lineage. In addition, in Figure 4 it is possible to identify individuals of other localities that were not included in this sampling within each lineage, therefore expanding their actual distribution. For example, individuals of La Lancha (located few km of CP) are added to lineage 1. Thus, *S. lomentaria* Chilean/European was restricted to three localities along the Southeastern Pacific coast. The lineage of *S. gracilis*, besides COQ, is also present in CACH, locality ~350 km away from COQ, but absent in between sites. The distribution of *Scytosiphon* sp. reached MB in Chiloe Island. And finally, *S. lomentaria* significantly expanded its range from Piquero (northern Chile) to Fuerte Bulnes in Punta Arenas. Besides the Chilean samples, also a Japanese sequences grouped with the *S. lomentaria* Chilean/Pacific clade. Another grouping composed of three Japanese sequences appeared as the most external of the *S. lomentaria* lineages. This topology suggests that at least part of the genetic diversity of Japanese *S. lomentaria* is related to Chilean lineages.

The geographic distribution of haplotypes between lineages was contrasting, although their diversity was not proportional to the size of their distribution range. For instance, *S. lomentaria* Chilean/European has the same number of haplotypes as *Scytosiphon* sp. (Nh = 9; Table 4), even though the former is restricted to two localities and the latter has a wide distribution along Chile (Figure 5). *S. lomentaria* Chilean/European displayed the highest number of haplotypes together with *Scytosiphon* sp. and the second highest diversity index despite having the most restricted geographical distribution (Hd = 0.792, π = 0.0029, Π = 1.616 ; Table 4,

Figure 5). *S. lomentaria* showed a reduced haplotype and nucleotide diversity, and pairwise nucleotide differences (Hd = 0.509, π = 0.0013, Π = 0.709; Table 4), despite the great number of sampled individuals (N=346). *S. gracilis* showed higher diversity indexes (Hd = 0.648, π = 0.0024, Π = 1.311; Table 4) despite the lower number of individuals analysed (N=49).

As in Figure 3, four well delineated groups, separated by more than 50 mutational steps, are identified in the network (Figure 6): green – *S. lomentaria* Chilean/European; red – *S. lomentaria*; blue – *Scytosiphon* sp. and yellow – *S. gracilis*. When analysing the relationships among haplotypes within each lineage we observed the following: for *S. lomentaria* Chilean/Pacific evidenced a dominant and widely distributed haplotype (H11), present along 1,330 Km, although not continuously (Figure 5). The absence of starlike structure of the network (Figure 6), as well as the Tajima and Fu and Li tests (D =0.229 p>0.10, Fu & Li = 0.845 p>0.10; Table 4) support an absence of recent demographic changes. The haplotype network of *Scytosiphon* sp. displayed highly divergent lineages (Figure 6), with mean Π = 4.116. For this species, H17 is the widest distributed, but only present in MAI, PUC and CAR, localities separated by 1,030 Km (Figure 5). *S. lomentaria* Chilean/European also displayed high haplotype diversity, with nine haplotypes detected in only two sites separated by 200m (Table 4 and Figure 5). No signature of recent demographic changes was detected from the network (Figure 6), as confirmed by the Tajima and Fu and Li tests (D = -0.961 p>0, Fu & Li = -1.473 p>0; Table 4). *S. gracilis* displayed high diversity despite its low geographic range of distribution (Table 4). The network showed that the haplotypes from COQ are associated through few mutational steps (Figure 6).

Finally, within each lineage the number of private haplotypes (those find in only one locality) are higher than the number of shared haplotypes (those found in two or more localities, not necessary neighbour localities, see Fig. 5). *Scytosiphon* sp. had 8 private haplotypes and 1 shared haplotype between MAI-PUC-CAR. The most extreme case occurs with *S. lomentaria* Chilean/European that presents seven private haplotypes in the only two sites where the species was observed in Chile, with 5 private alleles at CPC and 2 at CP (Figure 5).

4. DISCUSSION

This is the first comprehensive study that evaluates the genetic diversity of the species included in the genus *Scytosiphon* along the Southeastern Pacific coast. My analyses based on mitochondrial COX3 revealed a high level of genetic variation within the genus as evidenced by the recognition of four different lineages: *S. lomentaria*, *Scytosiphon* sp, *S. gracilis* and *S. lomentaria* Chilean/European. These lineages display contrasting geographic distributions. On one hand *S. lomentaria* and *Scytosiphon* sp. have a continuous distribution along the Southeastern Pacific coast, whereas on the other hand *S. gracilis* and *S. lomentaria* Chilean/European show a much more localized distribution in the region, the former present only in two separated localities and the later present only in two nearby sites. This pattern suggests different spatio-temporal origins for each of the lineages. Based on the results obtained, it is possible to suggest three main events to explain the occurrence of these lineages in the region. One possible event is a recent, likely human-induced introduction of *S. gracilis* and probably *S. lomentaria* Chilean/European. On the other hand, an ancient introduction likely explains the origin of *S. lomentaria*. Finally, a process of vicariance can be proposed for *Scytosiphon* sp., the only endemic lineage of the genus in the Southeastern Pacific coast. These scenarios are discussed in detail below.

S. gracilis in Chile, according to our study, is restricted to two localities 340 Km apart: COQ and CACH. Both populations are highly divergent, that could correspond to different species. The COQ population, despite its local distribution and low number of sampled individuals (N = 49), presents a particularly high haplotype diversity. However, for CACH population it is impossible to evaluate because of the

poor sampling effort. In the world, *S. gracilis* have been reported for the Pacific coast of Mexico (Aguilar-Rosas et al., 2006), Korea (Cho et al, 2002), Japan (Kogame, 1998) and CACH, Laguna Zapallar and MAI in central Chile (Contreras et al., 2007). Our reported distribution coincides with that of Contreras et al. (2007), who also suggest that Chilean *S. gracilis* correspond to the Korean-type based on nuclear ITS1 and ITS2 that present 99.8% and 100% of identity, respectively; the 25S ribosomal RNA gene that was 100% identical to the *S. gracilis* from Japan and the 5.8S rRNA, 99% identical to the Korean one. This suggests that a recent event of human-mediated introduction in CACH from Asia is a likely scenario. However, such scenario does not hold for the origin of the population of COQ, where high haplotype diversity and a high divergence among haplotypes were observed. *S. gracilis* in this location is either the result of multiple introductions or a long standing presence that allowed the emergence of private haplotypes. Indeed, population genetics theory predicts low genetic diversity in a population of recent colonizers (Holland, 2000). Multiple events of introductions to one locality can lead to high genetic diversity in terms of nucleotide diversity and divergence among haplotypes, and also, this depends of the genetic diversity of the source region, which in this case is unknown. Multiple introductions and/or large contingents of immigrants from a source region has been hypothesized for the brown algae *Undaria pinnatifida* and *Asparagopsis armata* to explain the substantial within-population genetic diversity discovered across the regions where they were introduced (Voisin et al., 2005; Andreakis et al., 2007). Likewise, the marine gastropod *Cyclope neritea* and the green crab *Carcinus maenas* shows high genetic diversity in its introduced range (Simon-Bouhet et al., 2006; Roman, 2006). Whatever the scenario for the origin of the high genetic diversity of *S.*

gracilis, comparisons with Asian and Mexican populations are needed to completely understand its origin.

S. lomentaria Chilean/European despite being restricted to two nearby sites in northern Chile, CP and CPC, showed higher haplotype diversity than *S. gracilis*. At this point, we must consider that these localities present certain particularities that may determine our results. Medina et al. (2005) reported elevated copper concentrations at CP and described this intertidal as characterized by bare rock (~80% cover) and four algal species, been *S. lomentaria* Chilean/European one of them (Camus et al., 2005). It is known that changes in the nutrient regime and/or anthropogenic disturbances are likely to make a habitat prone to invasion or provides resource opportunities to invaders since the residents may not be adapted to such resources fluctuations and changed environmental characteristics (Inderjit et al., 2006). However, as for *S. gracilis*, a recent invasion is possible only if multiple introductions are to be considered, as to explain the high diversity present in such a reduced species distribution. Alternatively, the effect of environmental conditions at CP, i.e. high copper concentrations (17.04 µg/L mean value of total dissolved copper concentration at CP (Medina et al., 2005) and reference values in unpolluted sites along the coast of northern Chile range from 0.5 to 6.5 µg/L (Correa et al., 2000)) can be invoked. There is abundant evidence that high copper levels in seawater result in toxicity to algal species (Gledhill et al., 1997). In particular, copper induces a strong oxidative stress in *S. lomentaria* (Contreras et al 2007). Recent findings showed that oxidative stress increases mutation rates of the mitochondrial genome (Lee and Wei, 2007), and ultimately that pollution can cause complex and rapid genetic changes in exposed populations within very short time scales (i.e. over a few generations) (Bickham et al., 2000; Gardestrom et al., 2008). This is a possible explanation of why

S. lomentaria Chilean/European remains restricted to CP, reaching higher percentages of cover at the middle intertidal zone (Camus et al., 2005). But how this species was able to arrive to these sites? Due to its high identity with the European *S. lomentaria* (87.7% with Rubisco spacer region and 93.7% with ITS1, Camus et al. 2005), it is possible to suggest an origin related to human-mediated introductions from Europe. But as for *S. gracilis*, such scenario implies that the diversity comes from European populations, which are currently understudied, and that a single massive or multiple introductions occurred in this single location. Here again, the main limitation is our poor knowledge of the potential source populations.

The type species of the genus, *S. lomentaria* present a wide distribution along the Southeastern Pacific coast: from Piquero (northern Chile) to Fuerte Bulnes (subantartic region), however low genetic diversity was evidenced. These results suggest a recent history in the region for this cosmopolitan species that rapidly colonized the whole actual distribution, as evidenced by the distribution of haplotype H11. This founder propagule probably originates from the Asia-Pacific because within the *S. lomentaria* clade we found high identity of Chilean individuals with one Japanese individual. However, few of the total genetic diversity of Japan is represented in Chile, because other Japanese individual form a highly support cluster that is separated from the Chilean *S. lomentaria* and even to the Chilean/European lineage. The Asiatic origin of the source propagule was also suggested in Chapter 1 based on plastidial and nuclear markers, where Chilean individuals grouped in the same clade as individuals from Korea, Japan, New Zealand and Australia. Therefore, it seems that, as opposed to *S. gracilis* and *S. lomentaria* Chilean/European, either a single introduction or a reduced subset of the original diversity was introduced in Chile. This fits the usual expectations of the genetics of introduction and invasion.

Finally, our results let us suggest the existence of a new species endemic to the Southeastern Pacific coasts distributed from MAI to MB in Chiloe Island, with high nucleotide diversity and nine haplotypes. Most of them appear restricted to one locality, although H17 is present in MAI-PUC and CAR. Thus, in order to occur in localities so distant from each other, at some point that haplotype should have colonized continuously the coast and then suffered local extinctions. Contrary to most other lineages/species that show a polytomy, *Scytosiphon* sp. shows a more resolved intraspecific phylogeny, with clearly basal haplotypes and more derived clades, indicating a more ancient or long lasting diversification. After detecting this new lineage, the main question that remains to be answered is how it originated in Chile? Our results and those presented in Chapter 1, suggest that *Scytosiphon* sp. originated in the region through a process of vicariance possibly involving *S. gracilis*. This hypothesis is supported by the tree topology (Figure 4). It is suggested that a founder event took place and strong genetic drift occurred resulting in genetic differentiation between both species in the newly colonized areas. Posterior to this event, *S. gracilis* was eradicated from the southern localities and *Scytosiphon* sp. was able to colonize these localities until Mar Brava located in Chiloe Island. To support the hypothesis of speciation of *Scytosiphon* sp.,it is required that *S. gracilis* has been present in the region for long time and actually is undergoing a process of disappearance in Chile, evidenced by it localize distribution and genetic diversity. This contrasts with the previous suggestion of an event of recent introductions to explain the origin of *S. gracilis* in Chile. To solve this discrepancy further studies are necessary.

Besides reporting contrasting genetic variation and geographical distribution of the *Scytosiphon* lineages, it is important to mention the high level of variation that

was detected within the type species of the genus, *S. lomentaria* in Chile. Both lineages (*S. lomentaria* and *S. lomentaria* Chilean/European) are separated by fifty-one fixed differences, which is sufficient to suggest different species if we consider, for example, that the number of fixed differences reported by Coyer et al. (2006) between species and lineages of *Fucus* spp. do not exceed 29 for a mitochondrial marker. Also Cho et al. (2007), based on nuclear and plastidial markers, recognized two independent clades within *S. lomentaria*, the Pacific and European clades. They suggest that both clades are separate species, however no morphological differences between Pacific and European entities were detected; therefore they preferred to refer as cryptic species. I agree with the proposal of Cho et al. (2007), but here we further infer that the occurrence of these genetically distinct lineages is the result of different events of introduction or immigration into the Southeastern Pacific coast. Accordingly, they correspond to cryptic species, morphologically equal but genetically different (Knowlton, 1993).

This study supports the view that phylogenetic and phylogeographic approximations contribute significantly to our understanding of algal biodiversity patterns, for which species presence/absence data clearly fades. Traditional measures of marine biodiversity greatly underestimated the true number of species principally because of the occurrence of sibling species, or species that are difficult to distinguish morphologically. In addition, I have shown here that the distribution pattern is not always a good indicator of the status of a species (i.e. native, endemic or introduced). Indeed, high genetic diversity could be observed even in very narrow distribution ranges. Except in the case of *S. lomentaria* Chilean/European, whose diversity can result from an increased mutation rate associated to stressful condition of the copper

pollution, the case of *S. gracilis* is particularly awkward. This species is either introduced from multiple or highly diverse origins or it was present in Chile since ancient times. The main doubt here was introduced by the evolutionary relationship of this taxon with *Sytosiphon* sp. Because of that, it is not enough to just identify and enumerate species. The evolutionary relationships among them must be understood. And in the case of the *Scytosiphon* genus, a better knowledge of the genetic diversity and the evolutionary relationships among lineages and regions of the world is required. This approximation is especially relevant in phycology, because of the general complex and ancient evolutionary histories and lack of traditional morphological diagnostic characters. Such studies should provide a basis for predicting for example, biodiversity responses to environmental changes, the spread of lineages into new areas, and the factors that have shaped the composition of regional species pools and local communities (Hendry et al. 2010).

5. ACKNOWLEDGMENTS

This research was funded by FONDAP 1501-0001 (CONICYT) and the Center for Advanced Studies in Ecology and Biodiversity (CASEB) Program 7. C.Camus received a doctoral grant from CONICYT. I sincerely thank Jessica Beltrán and Javier Infante for collecting specimens and assisting in the field, also Gioconda Peralta, Martha Hengst and Florence Tellier for their invaluable help with the SSCP.

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TABLE 1: Geographic location of collection sites used in this study.

Collection site and date	Abbreviation	Coordinates (Latitude; Longitude)	External morphology	N
Caleta Palito 200; 14 July 2007	CP	26°15,79´S; 70°40,63´W	Constriction	23
Caleta Palito canal; 14 July 2007	CPC	26°15,79´S; 70°40,63´W	Constriction	30
Coquimbo 1; 30 July 2007	COQ	29°56,108´S; 71°20,168´W	Cylindrical	26
Coquimbo 2; 30 July 2007	COQ	29°56,108´S; 71°20,168´W	Flat	25
Coquimbo 3: 30 July 2007	COQ	29°56,108´S; 71°20,168´W	Constriction	29
Pichidangui 1; 29 July 2007	PI	32°09,462´S; 71°31,89´W	Constriction	30
Pichidangui 2; 29 July 2009	PI	32°09,462´S; 71°31,89´W	Cylindrical	27
Maitencillo; 5 November 2007	MAI	32°39,319´S; 71°26,642´W	Constriction	17
El Tabo; 28 July 2007	TABO	33°27,562´S; 71°39,810´W	Constriction	26
Las Cruces; 28 July 2007	LC	33°30,165´S; 71°37,976´W	Constriction	30
Pelancura; 7 November 2007	PE	33°33,420´S; 71°37,595´W	Constriction	26
Curanipe; 26 October 2007	CUR	35°50´30,0´´S; 72°38´20,7´´W	Constriction	30
Cocholgue 1; 25 October 2007	COCH	36°36´26,4´´S; 72°58´48,9´´W	Flat	26
Cocholgue 2; 25 October 2007	COCH	36°36´26,4´´S; 72°58´48,9´´W	Constriction	30
Lebu 1; 24 October 2007	LEBU	37°34´47,3´´S; 73°38´32,0´´W	Constriction	28
Lebu 2; 24 October 2007	LEBU	37°34´47,3´´S; 73°38´32,0´´W	Flat	28
Niebla; 23 October 2007	NIE	39°52,465´S; 73°24,017´W	Flat	28
Pucatrihue 1; 22 October 2007	PUCA	40°32,812´S; 73°43,150´W	Constriction	17
Pucatrihue 2; 22 October 2007	PUCA	40°32,812´S; 73°43,150´W	Thin	18
Carelmapu 1; 21 October 2007	CAREL	41°44,465´S; 73°44,113´W	Constriction	28
Carelmapu 2; 21 October 2007	CAREL	41°44,465´S; 73°44,113´W	Flat	28
Carelmapu 3; 21 October 2007	CAREL	41°44,465´S; 73°44,113´W	Thin	30
				N=580

N: number of individuals used for analysis.

TABLE 2: Collection site and GenBank accession number of published sequences used in this study.

Collection site	GenBank accesion number	Reference
S .lomentaria Caleta Palito	GU252561	Chapter 1
S. lomentaria Caleta Palito canal	GU252560	Chapter 1
S. lomentaria La Lancha	GU252563	Chapter 1
S. lomentaria La Lancha	GU252562	Chapter 1
S. lomentaria Piquero	GU252592	Chapter 1
S. lomentaria Coquimbo	GU252569	Chapter 1
S. lomentaria Coquimbo	GU252591	Chapter 1
S. lomentaria Pichidangui	GU252568	Chapter 1
S. lomentaria Cachagua	GU252573	Chapter 1
S. lomentaria Maitencillo	GU252575	Chapter 1
S. lomentaria Reñaca	GU252585	Chapter 1
S. lomentaria La Boca	GU252586	Chapter 1
S. lomentaria Algarrobo	GU252580	Chapter 1
S. lomentaria Algarrobo	GU252581	Chapter 1
S. lomentaria El Quisco	GU252582	Chapter 1
S. lomentaria El Tabo	GU252584	Chapter 1
S. lomentaria Las Cruces	GU252572	Chapter 1
S. lomentaria Pelancura	GU252566	Chapter 1
S. lomentaria Pelancura	GU252576	Chapter 1
S. lomentaria Matanza	GU252587	Chapter 1
S. lomentaria Curanipe	GU252595	Chapter 1
S. lomentaria Cocholgue	GU252594	Chapter 1
S. lomentaria Lebu	GU252596	Chapter 1
S. lomentaria Pucatrihue	GU252565	Chapter 1
S. lomentaria Punta Estaquilla	GU252577	Chapter 1
S. lomentaria Carelmapu	GU252578	Chapter 1
S. lomentaria Mar Brava	GU252598	Chapter 1
S. lomentaria Cucao	GU252571	Chapter 1
S. lomentaria Fuerte Bulnes	GU252564	Chapter 1
S. lomentaria Isla Carlos III	GU252597	Chapter 1
S. lomentaria Japan	AB094194	Kogame et al. 2005
S. lomentaria Japan	AB094195	Kogame et al. 2005
S. lomentaria Japan	AB094196	Kogame et al. 2005
S. lomentaria Japan	AB094197	Kogame et al. 2005
S. gracilis Coquimbo	GU252622	Chapter 1
S. gracilis Cachagua	GU252623	Chapter 1
Scytosiphon sp. Maitencillo	GU252619	Chapter 1
Scytosiphon sp. Reñaca	GU252615	Chapter 1
Scytosiphon sp. El Tabo	GU252606	Chapter 1
Scytosiphon sp. Las Cruces	GU252604	Chapter 1

Scytosiphon sp. Las Cruces	GU252618	Chapter 1
Scytosiphon sp. Pelancura	GU252603	Chapter 1
Scytosiphon sp. Matanza	GU252609	Chapter 1
Scytosiphon sp. Cocholgue	GU252616	Chapter 1
Scytosiphon sp. Niebla	GU252611	Chapter 1
Scytosiphon sp. Niebla	GU252612	Chapter 1
Scytosiphon sp. Niebla	GU252621	Chapter 1
Scytosiphon sp. Pucatrihue	GU252614	Chapter 1
Scytosiphon sp. Pucatrihue	GU252607	Chapter 1
Scytosiphon sp. Punta Estaquilla	GU252610	Chapter 1
Scytosiphon sp. Carelmapu	GU252613	Chapter 1
Scytosiphon sp. Carelmapu	GU252620	Chapter 1
Scytosiphon sp. Carelmapu	GU252617	Chapter 1
Scytosiphon sp. Mar Brava	GU252608	Chapter 1
S. fascia Cachagua	GU252639	Chapter 1
S. fascia Maitencillo	GU252640	Chapter 1
S. fascia Mar Brava	GU252641	Chapter 1
S. fascia Pucatrihue	GU252646	Chapter 1
S. fascia Carelmapu	GU252642	Chapter 1
S. fascia Pucatrihue	GU252643	Chapter 1
S. fascia Las Cruces	GU252644	Chapter 1
S. fascia Carelmapu	GU252645	Chapter 1
S. fascia Carelmapu	GU252647	Chapter 1
S. fascia Las Cruces	GU252648	Chapter 1
S. fascia Punta Estaquilla	GU252638	Chapter 1
S. fascia Cocholgue	GU252650	Chapter 1
S. fascia Lebu	GU252651	Chapter 1
S. binghamiae Paposo	GU252624	Chapter 1
S. binghamiae Arica	GU252625	Chapter 1
S. binghamiae Reñaca	GU252626	Chapter 1
S. binghamiae Matanza	GU252627	Chapter 1
S. binghamiae Pelancura	GU252628	Chapter 1
S. binghamiae Playa Los Verdes	GU252629	Chapter 1
S. binghamiae Algarrobo	GU252639	Chapter 1
S. binghamiae Caleta Cobija	GU252631	Chapter 1
S. binghamiae Caleta Chipana	GU252632	Chapter 1
S. binghamiae Las Cruces	GU252633	Chapter 1
S. binghamiae Cachagua	GU252634	Chapter 1
S. binghamiae Caleta Constitución	GU252635	Chapter 1
S. binghamiae Coquimbo	GU252636	Chapter 1
S. binghamiae Punta Choros	GU252637	Chapter 1
S. binghamiae La Boca	GU252638	Chapter 1
S. tenellus Caleta Palito	GU252553	Chapter 1
Pylaiella littoralis	AJ277126	Outdot-Le Secq et al. 2001

TABLE 3: Number of fixed differences between the *Scytosiphon* lineages.

	S. lomentaria	*S. lomentaria* Chilean/European	*Scytosiphon* sp.	*S. gracilis*
S. lomentaria	0			
S. lomentaria Chilean/European	51	0		
Scytosiphon sp.	61	53	0	
S. gracilis	77	80	66	0

TABLE 4: Diversity measures for *Scytosiphon* lineages.

Lineages	N	Nh	S	Hd	π	Π	D Tajima	Fu and Li
S. lomentaria	346	6	4	0.509	0.0013	0.709	0.229 P>0.10	0.845 P>0.10
S. lomentaria Chilean/European	53	9	10	0.792	0.0029	1.616	-0.961 P>0.10	-1.473 P>0.10
Scytosiphon sp.	128	9	18	0.833	0.0075	4.116	0.665 P>0.10	1.182 P>0.10
S. gracilis	49	4	5	0.648	0.0024	1.311	0.412 P>0.10	1.098 P>0.10
Total	**576**	**28**						

N: number of individuals, Nh: number of haplotypes, S: segregating sites, Hd: haplotype diversity, π: nucleotide diversity, Π: average number of nucleotide differences.

FIGURE LEGENDS

Figure 1. Map of Chile showing the collection sites.

Figure 2. Alignment of COX3 haplotypes. Only polymorphic sites are shown and the sequences are grouped by similarity.

Figure 3. Maximum likelihood tree based on COX3 haplotypes. Support values are presented as maximum likelihood bootstrap (n=1000). Abbreviations as Table 1.

Figure 4. Maximum Likelihood tree based on COX3 sequences. Both ML and BI trees were congruent, therefore ssupport values are presented as maximum likelihood bootstrap (n=1000) and Bayesian posterior probability, respectively, and – indicates <50% of support.

Figure 5. Geographic distribution of the COX3 haplotypes of *Scytosiphon* lineages. The color of the circles represents the different lineages: orange corresponds to *S. gracilis*, blue to *Scytosiphon* sp., green to *S. lomentaria* Chilean/European, and red to *S. lomentaria*. The size of the circles is proportional to the number of individuals analysed in each location (2 to 57).

Figure 6. Haplotype network based on COX3 haplotypes of *Scytosiphon* lineages. In green, *S. lomentaria* Chilean/European; red, *S. lomentaria*; blue, *Scytosiphon* sp. and yellow, *S. gracilis*. In parenthesis the number of individuals (N) per haplotype and the symbol * indicates the number of mutational steps between haplotypes.

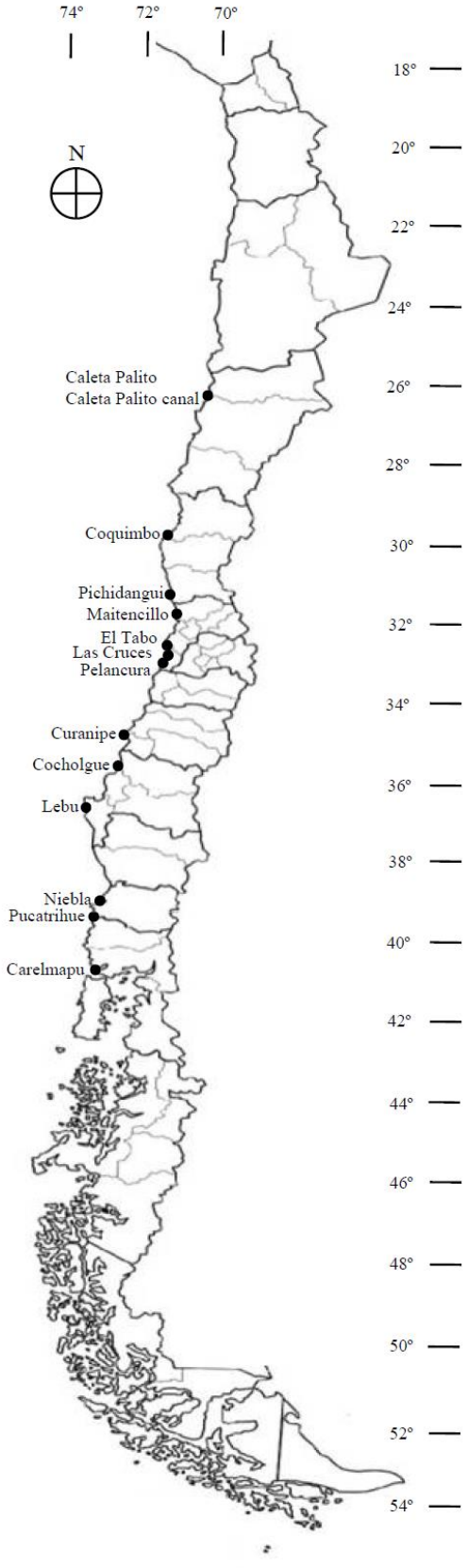
74°
72°
70°
N
18°
20°
22°
24°
26°
28°
30°
32°
34°
36°
38°
40°
42°
44°
46°
48°
50°
52°
54°
Caleta Palito
Caleta Palito canal
Coquimbo
Pichidangui
Maitencillo
El Tabo
Las Cruces
Pelancura
Curanipe
Cocholgue
Lebu
Niebla
Pucatrihue
Carelmapu

Figure 1.

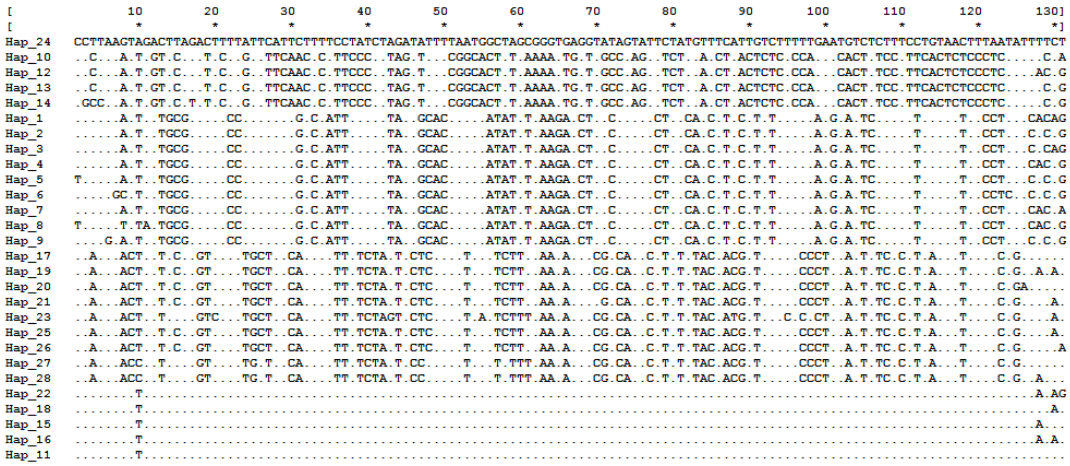
[10 20 30 40 50 60 70 80 90 100 110 120 130]
Hap_24 CCTTAAGTAGACTTAGACTTTTATTCATTCTTTTCCTATCTAGATATTTTAATGGCTAGCGGGTGAGGTATAGTATTCTATGTTTCATTGTCTTTTTGAATGTCTCTTTCCTGTAACTTTAATATTTTCT
Hap_10 ..C...A.T.GT.C...T.C..G..TTCAAC.C.TTCCC..TAG.T...CGGCACT.T.AAAA.TG.T.GCC.AG..TCT..A.CT.ACTCTC.CCA...CACT.TCC.TTCACTCTCCCTC.....C.A
Hap_12 ..C...A.T.GT.C...T.C..G..TTCAAC.C.TTCCC..TAG.T...CGGCACT.T.AAAA.TG.T.GCC.AG..TCT..A.CT.ACTCTC.CCA...CACT.TCC.TTCACTCTCCCTC....AC.G
Hap_13 ..C...A.T.GT.C...T.C..G..TTCAAC.C.TTCCC..TAG.T...CGGCACT.T.AAAA.TG.T.GCC.AG..TCT..A.CT.ACTCTC.CCA...CACT.TCC.TTCACTCTCCCTC.....C.G
Hap_14 .GCC..A.T.GT.C.T.T.C..G..TTCAAC.C.TTCCC..TAG.T...CGGCACT.T.AAAA.TG.T.GCC.AG..TCT..A.CT.ACTCTC.CCA...CACT.TCC.TTCACTCTCCCTC.....C.G
Hap_1 ......A.T..TGCG.....CC.......G.C.ATT.....TA..GCAC.....ATAT.T.AAGA.CT..C.....CT..CA.C.T.C.T.T.....A.G.A.TC.....T.....T..CCT...CACAG
Hap_2 ......A.T..TGCG.....CC.......G.C.ATT.....TA..GCAC.....ATAT.T.AAGA.CT..C.....CT..CA.C.T.C.T.T.....A.G.A.TC.....T.....T..CCT...C.C.G
Hap_3 ......A.T..TGCG.....CC.......G.C.ATT.....TA..GCAC.....ATAT.T.AAGA.CT..C.....CT..CA.C.T.C.T.T.....A.G.A.TC.....T.....T..CCT...C.CAG
Hap_4 ......A.T..TGCG.....CC.......G.C.ATT.....TA..GCAC.....ATAT.T.AAGA.CT..C.....CT..CA.C.T.C.T.T.....A.G.A.TC.....T.....T..CCT...CAC.G
Hap_5 T.....A.T..TGCG.....CC.......G.C.ATT.....TA..GCAC.....ATAT.T.AAGA.CT..C.....CT..CA.C.T.C.T.T.....A.G.A.TC.....T.....T..CCT...C.C.G
Hap_6 .....GC.T..TGCG.....CC.......G.C.ATT.....TA..GCAC.....ATAT.T.AAGA.CT..C.....CT..CA.C.T.C.T.T.....A.G.A.TC.....T.....T..CCTC..C.C.G
Hap_7 ......A.T..TGCG.....CC.......G.C.ATT.....TA..GCAC.....ATAT.T.AAGA.CT..C.....CT..CA.C.T.C.T.T.....A.G.A.TC.....T.....T..CCT...CAC.A
Hap_8 T.....T.TA.TGCG.....CC.......G.C.ATT.....TA..GCAC.....ATAT.T.AAGA.CT..C.....CT..CA.C.T.C.T.T.....A.G.A.TC.....T.....T..CCT...CAC.G
Hap_9 ....G.A.T..TGCG.....CC.......G.C.ATT.....TA..GCAC.....ATAT.T.AAGA.CT..C.....CT..CA.C.T.C.T.T.....A.G.A.TC.....T.....T..CCT...C.C.G
Hap_17 ..A...ACT..T.C..GT....TGCT..CA....TT.TCTA.T.CTC....T...TCTT..AA.A...CG.CA..C.T.T.TAC.ACG.T.....CCCT..A.T.TC.C.T.A...T....C.G......
Hap_19 ..A...ACT..T.C..GT....TGCT..CA....TT.TCTA.T.CTC....T...TCTT..AA.A...CG.CA..C.T.T.TAC.ACG.T.....CCCT..A.T.TC.C.T.A...T....C.G..A.A.
Hap_20 ..A...ACT..T.C..GT....TGCT..CA....TT.TCTA.T.CTC....T...TCTT..AA.A...CG.CA..C.T.T.TAC.ACG.T.....CCCT..A.T.TC.C.T.A...T....C.GA.....
Hap_21 ..A...ACT..T.C..GT....TGCT..CA....TT.TCTA.T.CTC....T...TCTT..AA.A....G.CA..C.T.T.TAC.ACG.T.....CCCT..A.T.TC.C.T.A...T....C.G....A.
Hap_23 ..A...ACT..T....GTC...TGCT..CA....TT.TCTAGT.CTC....T.A.TCTTT.AA.A...CG.CA..C.T.T.TAC.ATG.T...C.C.CT..A.T.TC.C.T.A...T....C.G....A.
Hap_25 ..A...ACT..T.C..GT....TGCT..CA....TT.TCTA.T.CTC....T...TCTT..AA.A...CG.CA..C.T.T.TAC.ACG.T.....CCCT..A.T.TC.C.T.A...T....C.G....A.
Hap_26 ..A...ACT..T.C..GT....TGCT..CA....TT.TCTA.T.CTC....T...TCTT..AA.A...CG.CA..C.T.T.TAC.ACG.T.....CCCT..A.T.TC.C.T.A...T....C.G.....A
Hap_27 ..A...ACC..T....GT....TG.T..CA....TT.TCTA.T.CC.....T...T.TTT.AA.A...CG.CA..C.T.T.TAC.ACG.T.....CCCT..A.T.TC.C.T.A...T....C.G......
Hap_28 ..A...ACC..T....GT....TG.T..CA....TT.TCTA.T.CC.....T...T.TTT.AA.A...CG.CA..C.T.T.TAC.ACG.T.....CCCT..A.T.TC.C.T.A...T....C.G..A...
Hap_22 ........T.....A.AG
Hap_18 ........T.....A.
Hap_15 ........T.....A...
Hap_16 ........T.....A.A.
Hap_11 ....T.....

Figure 2.

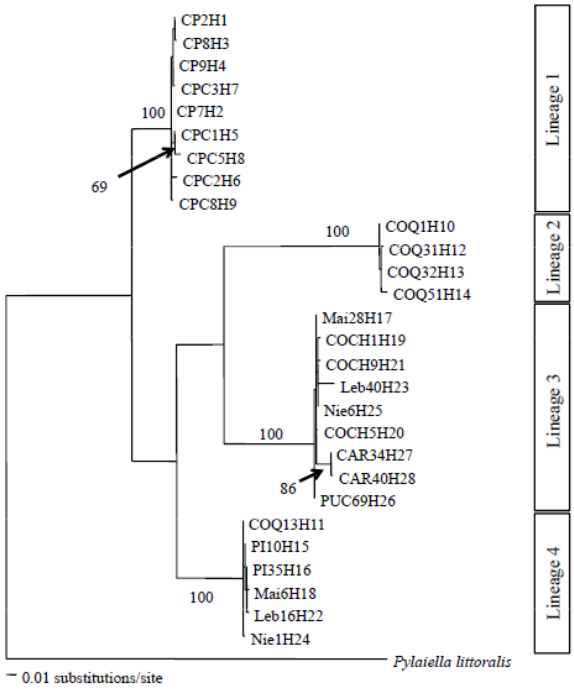
CP2H1
CP8H3
CP9H4
CPC3H7
100
CP7H2
CPC1H5
CPC5H8
69
CPC2H6
CPC8H9
100
COQ1H10
COQ31H12
COQ32H13
COQ51H14
Mai28H17
COCH1H19
COCH9H21
Leb40H23
Nie6H25
100
COCH5H20
CAR34H27
CAR40H28
86
PUC69H26
COQ13H11
PI10H15
PI35H16
100
Mai6H18
Leb16H22
Nie1H24
Pylaiella littoralis
0.01 substitutions/site
Lineage 1
Lineage 2
Lineage 3
Lineage 4

Figure 3.

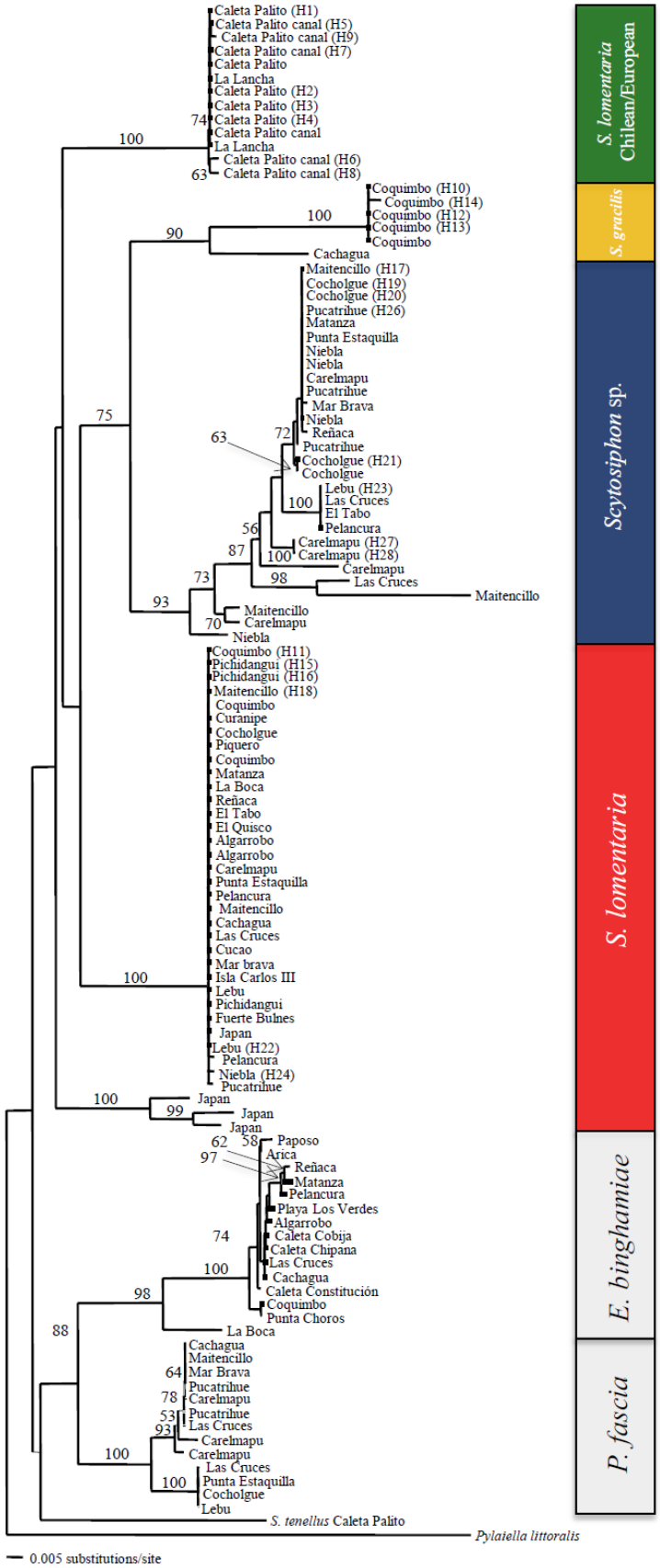
Caleta Palito (H1)
Caleta Palito canal (H5)
Caleta Palito canal (H9)
Caleta Palito canal (H7)
Caleta Palito
La Lancha
Caleta Palito (H2)
Caleta Palito (H3)
Caleta Palito (H4)
Caleta Palito canal
La Lancha
Caleta Palito canal (H6)
Caleta Palito canal (H8)
Coquimbo (H10)
Coquimbo (H14)
Coquimbo (H12)
Coquimbo (H13)
Coquimbo
Cachagua
Maitencillo (H17)
Cocholgue (H19)
Cocholgue (H20)
Pucatrihue (H26)
Matanza
Punta Estaquilla
Niebla
Niebla
Carelmapu
Pucatrihue
Mar Brava
Niebla
Reñaca
Pucatrihue
Cocholgue (H21)
Cocholgue
Lebu (H23)
Las Cruces
El Tabo
Pelancura
Carelmapu (H27)
Carelmapu (H28)
Carelmapu
Las Cruces
Maitencillo
Maitencillo
Carelmapu
Niebla
Coquimbo (H11)
Pichidangui (H15)
Pichidangui (H16)
Maitencillo (H18)
Coquimbo
Curanipe
Cocholgue
Piquero
Coquimbo
Matanza
La Boca
Reñaca
El Tabo
El Quisco
Algarrobo
Algarrobo
Carelmapu
Punta Estaquilla
Pelancura
Maitencillo
Cachagua
Las Cruces
Cucao
Mar brava
Isla Carlos III
Lebu
Pichidangui
Fuerte Bulnes
Japan
Lebu (H22)
Pelancura
Niebla (H24)
Pucatrihue
Japan
Japan
Japan
Paposo
Arica
Reñaca
Matanza
Pelancura
Playa Los Verdes
Algarrobo
Caleta Cobija
Caleta Chipana
Las Cruces
Cachagua
Caleta Constitución
Coquimbo
Punta Choros
La Boca
Cachagua
Maitencillo
Mar Brava
Pucatrihue
Carelmapu
Pucatrihue
Las Cruces
Carelmapu
Carelmapu
Las Cruces
Punta Estaquilla
Cocholgue
Lebu
S. tenellus Caleta Palito
Pylaiella littoralis
0.005 substitutions/site
S. lomentaria Chilean/European
S. gracilis
Scytosiphon sp.
S. lomentaria
E. binghamiae
P. fascia

Figure 4.

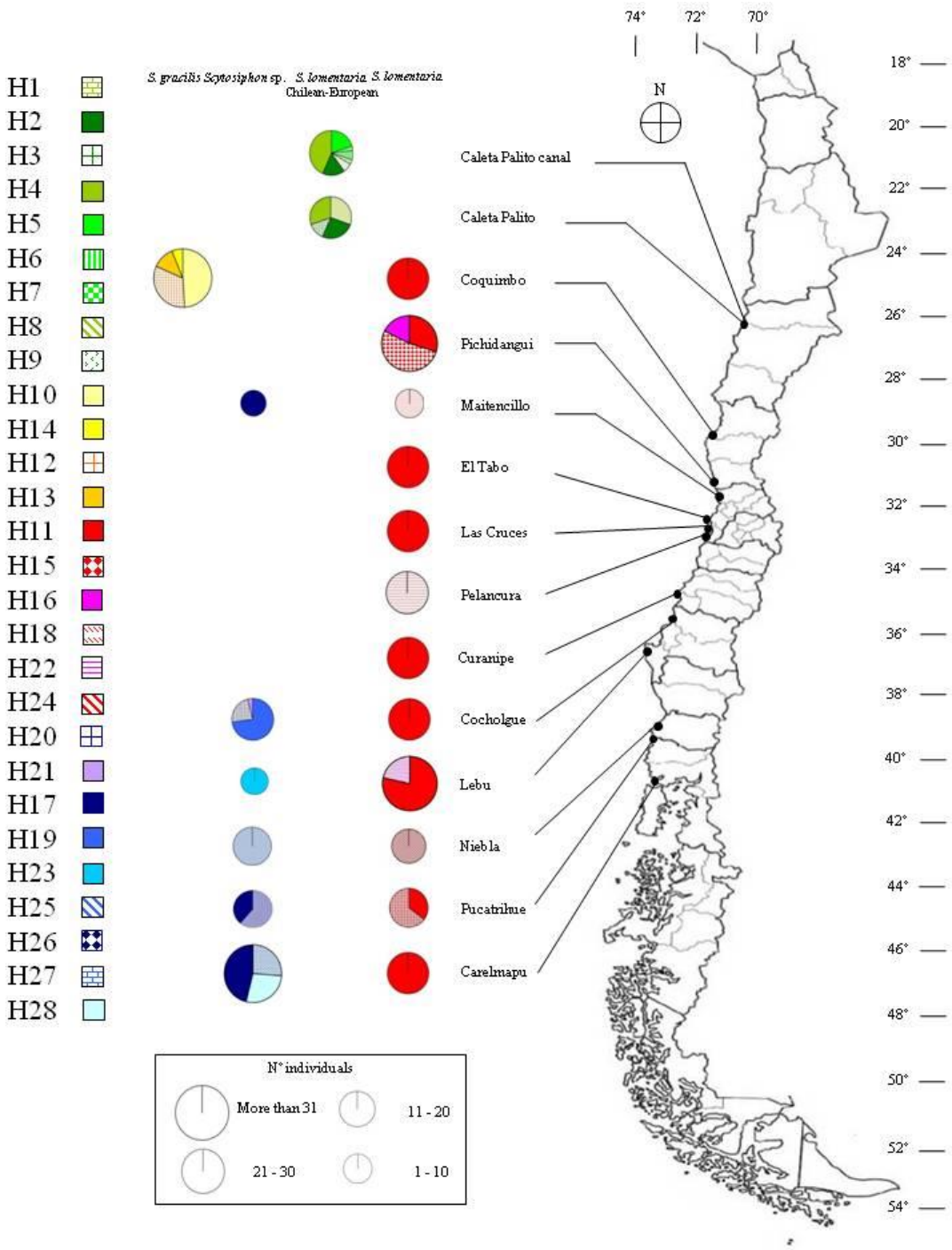
H1
H2
H3
H4
H5
H6
H7
H8
H9
H10
H14
H12
H13
H11
H15
H16
H18
H22
H24
H20
H21
H17
H19
H23
H25
H26
H27
H28
S. gracilis Scytosiphon sp.
S. lomentaria Chilean-European
S. lomentaria
74°
72°
70°
N
18°
20°
22°
24°
26°
28°
30°
32°
34°
36°
38°
40°
42°
44°
46°
48°
50°
52°
54°
Caleta Palito canal
Caleta Palito
Coquimbo
Pichidangui
Maitencillo
El Tabo
Las Cruces
Pelancura
Curanipe
Cocholgue
Lebu
Niebla
Pucatrihue
Carelmapu
N° individuals
More than 31
11 - 20
21 - 30
1 - 10

Figure 5.

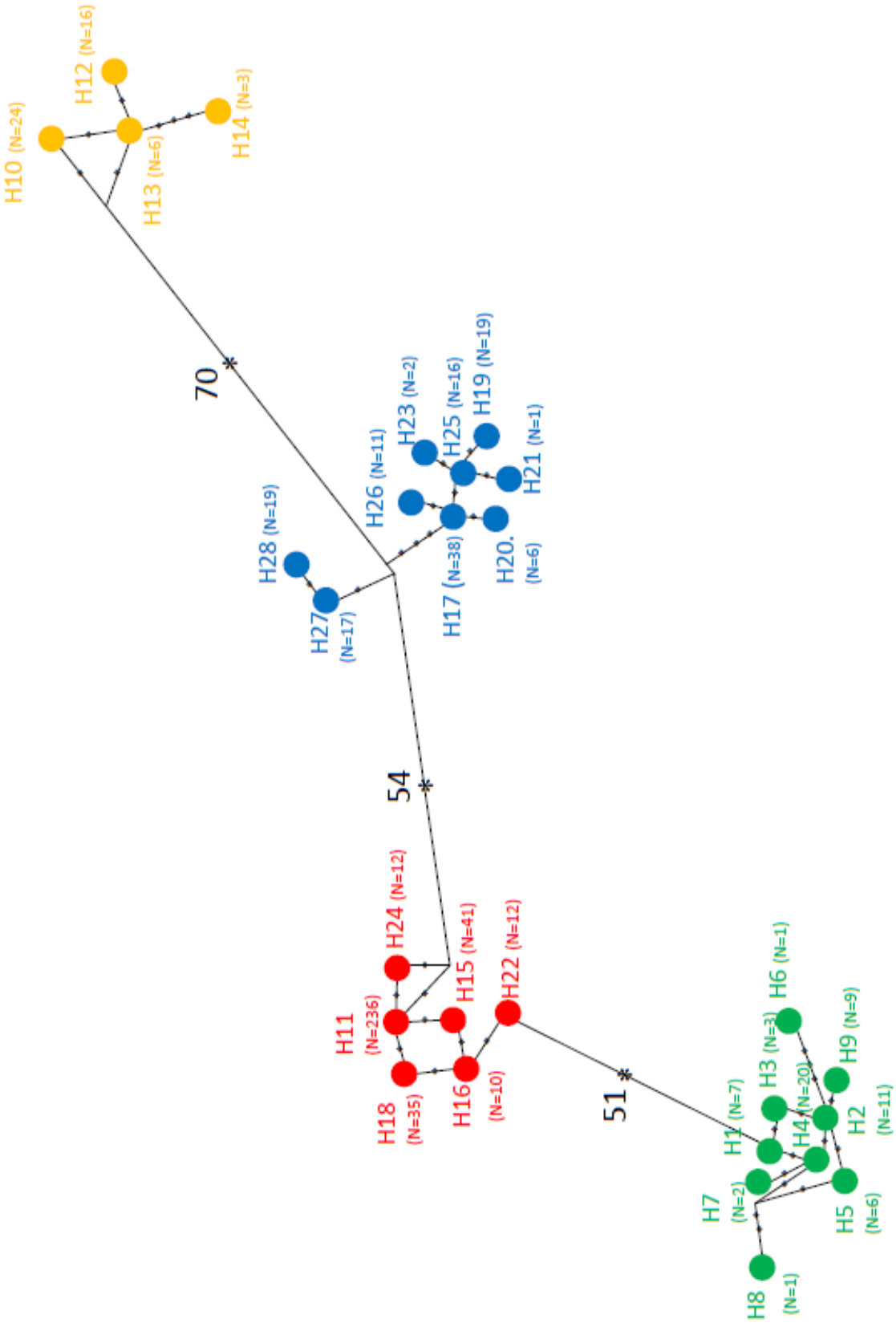
H10 (N=24)
H12 (N=16)
H14 (N=3)
H13 (N=6)
70 *
H23 (N=2)
H25 (N=16)
H19 (N=19)
H26 (N=11)
H21 (N=1)
H28 (N=19)
H20. (N=6)
H17 (N=38)
H27 (N=17)
54 *
H24 (N=12)
H15 (N=41)
H22 (N=12)
H11 (N=236)
H18 (N=35)
H16 (N=10)
51 *
H6 (N=1)
H3 (N=3)
H9 (N=9)
H1 (N=7)
H4 (N=20)
H2 (N=11)
H7 (N=2)
H5 (N=6)
H8 (N=1)

Figure 6

GENERAL DISCUSSION

This research has given important insights into the taxonomy and history of the Scytosiphonaceae family in the Southeastern Pacific coast. The study of DNA sequences (nuclear, mitochondrial and chloroplastidial) together with morphological data revealed more diversity than expected within the genus involved, and also different processes responsibly for the origin of some of them. Particularly for the *Scytosiphon* genus, this study provides evidence that clarifies the historical and contemporaneous processes that shaped the genus in the Southeastern Pacific region.

Traditionally *S. lomentaria*, a cosmopolitan and worldwide distributed species, was recognized as the only representative of the genus in the Southeastern Pacific coast, distributed from Antofagasta to Cape Horn. However, after this study, we achieved a better understanding of the species and genus that allowed us to recognize four lineages within what was supposed to be one entity. This is not an isolated case for seaweeds species, nor is it in the region. The case of the *Lessonia nigrescens* species complex in Chile (Tellier et al., 2009; 2011a; 2011b), and *Durvillaea* complex in Chile/N. Zealand/Australia (Fraser et al., 2009a; 2009b) are examples that genetic, morphological, and ecological-based approximations sometimes underestimate species diversity. Also, the genetic data is demonstrating that the factors acting over the genus/species are, in most cases, associated to the biogeography of the region (i.e. *D. antartica* (Fraser et al., 2009a) and *L. nigrescens* species complex (Tellier et al., 2009)). For the genus *Durvillaea*, recent morphological and molecular work demonstrated that the genus includes more than one species and at least four lineages within the range of distribution of *D. antartica* (recent addition of *D. poha* sp. nov.), distinguished by a north-south phylogeographic break between 36°S and 39°S (Fraser et al., 2009; 2012).

In the case of *L. nigrescens*, a similar situation occurs: a phylogenetic study revealed two strongly divergent lineages within the species which was traditionally described as one entity distributed along the Chilean coast. One species is located between 16°S and 30°S, and the second is present from 29°S to 42°S without hybridization detected between both (Tellier et al., 2009 and 2011a), reinforcing the argument that they correspond to two different species. For *Scytosiphon* genus the situation is different; two contrasting patterns of distribution are observed - lineages with restricted distribution (*S. gracilis*, *S. lomentaria* Chilean/European and *S. tenellus*) and lineages widely distributed (*S. lomentaria* Pacific and *Scytosiphon* sp.). Of these last, the first one crosses both biogeographic breaks described for the Southeastern Pacific Coast, with no genetic differentiation from Piquero (26°S) to Fuerte Bulnes (53°S) and the second one remains retained within the Intermediate Area between Maitencillo - 32°S and Mar Brava – 41°S in Chiloe Island. The comparison of the distribution of this genus and the others mentioned above is not strait forward because the data suggest that the factors that modulate the distribution of the genetic diversity in the region are different, for *Scytosiphon* genus, contemporary events of introductions are the most plausible explanation to the observed pattern versus environmental adaptation (*L. nigrescens* complex) and Last Glacial Maximum (LGM) (*D. antartica* complex) that have been claimed for the other studied species. The exception to the pattern is *Scytosiphon* sp. which is suggested to have evolved in the region through an event of vicariance and not by introduction. More information is necessary to address the reasons of its actual distribution, however one possible scenario to explained its southern limit (mar brava – 41°S), is the LGM that eliminated the most austral populations of *Scytosiphon* sp., limiting its distribution up to 41°S, which coincide with the estimated extension of the ice sheet (McCulloch et al., 2000), and after this, the species was unable to recolonize

again higher latitudes. Despite we don´t have conclusive data to support this suggestion, it is proposed because other studies on seaweed of the area (Tellier et al., 2009, Fraser et al., 2009, Macaya and Zuccarello, 2010) have shown that the LGM is a common feature in structuring the genetic diversity among them. On the other hand, the northern limit (32°S) of this species is more difficult to explain because as far as we know, no particular oceanographic or ecological event is associated with the Maitencillo area.

Following the same logic above, for *S. lomentaria* Pacific, one could also explain its distribution by invoking the LGM, but in this case, after the retreat of the ice, a process of recolonization from glacial refuge can be suggested. This would be consistent with the low diversity detected in the group and the wide distribution of a dominant haplotype, the one that may have colonized.

For the other two lineages, with restricted distributions, the data allows us to suggest that they originated in the region by recent events of introductions. However, it is difficult to explain why they remain in their localities a show no signal of colonization to other areas, and maintained higher genetic diversity. As mention in Chapter 1, here the main limitation is our poor knowledge of the potential source populations.

As mentioned before and also in the general introduction, an increasing number of cryptic species have been recently revealed using different molecular approaches: molecular sequences of different genomic compartments (nucleus, mitochondria and chloroplasts) or barcoding. Barcoding is a useful tool to discover cryptic diversity, in particular for Laminariaceae (McDevitt and Saunders, 2010). However, barcoding alone has to be taken carefully because current techniques, usually based on the use of only one molecular marker, could lead to confusion between evolutionary history of the

species and that of the molecular marker. In this thesis, the combination of three molecular markers of different genome was considered to confirm the existence of the four lineages within the *Scytosiphon* genus, together with a dedicated sampling along the coast (one sampling point in each degree of latitude, i.e. no more than 100 km away) to determine the distribution of lineages and study of the internal and external morphology. With this information we showed the problem that arises when attempting to delimit species based on only one type of information. This is similar to using one species concept for delimiting species, it leads to the generation of conflict because it makes the delimitation of species dependent of the definition of species that the authors select. Each definition includes different perspectives of what a species is. Most commonly authors define species based on one of three concepts: Biological species concept (BSC), Phylogenetic species concept (PSC) and Ecological species concept (ESC). However, as most species descriptions conform to what can be regarded as the typological or morphological species concept (TSC), this definition, at least in seaweeds, is one of the most used. This thesis shows that this is a mistake, at least in groups of complex species. For example, the case of the genus *Macrocystis* used to consider four species based on morphological characters of the holdfast, now, the actual evidence (molecular (PSC) (Macaya, 2000), ecological (ESC) (Demes et al., 2009), and hybridization (BSC) (Westermeier et al., 2009)) support that the genus is monotypic, composed only by *M. pyrifera*. This indicates that to define complex groups of species, it is not possible to consider one concept only. Moreover, when integrating both chapters of this thesis, the importance of multicharacter approach arises, taking into account the evolutionary history of the entities (phylogenies), and the embedded biogeographic history. This not only confirms that there are distinct lineages following independent evolutionary process, but also helps to understand the mechanisms that are

explaining the observed diversity and eventually how it's being maintained. Last but not least, I also emphasize that morphological data, together with the other approaches, is relevant; but how to address them should be reconsidered. In Chapter 1 I show that each character by itself does not allow discriminating species, however, the use of all of them in the same multivariate analysis shows another dimension that allows finding a combination of characters that contribute to the separation of species or lineages of complex groups such as *Scytosiphon*. I emphasize this because the morphological characterization is traditionally essential when working with species of macroalgae (because they are still defined based on the typological concept) and particularly with groups of cryptic species, because it is assumed that they result from a process of recent speciation where morphological features or other diagnostic characters have not evolved enough to differentiate (Bickford et al., 2006).

Finally, the identification and description of cryptic species have important implications for conservation, natural resource protection and management. The unexpected diversity discovered within the family Scytosiphonaceae in the Southestearn Pacific coast is relevant from this point of view. However, beyond the great diversity reported, it is necessary to realize that three of the four lineages recognized in this thesis correspond to introductions that successfully colonized particular areas and only one correspond to an endemic species. The implications of these events for conservation of natural resources of the region are relevant. For example, the case of *S. lomentaria* Chilean/European is one of the few species able to survive in a heavy metal contaminated area (C. Palito/La Lancha). No information is available about the diversity of C. Palito before the contamination event, but probably *S. lomentaria* was present in the area because is a common entity described along the intertidal from Antofagasta to

Cape Horn. The arrival of this introduced entity may have outcompeted the former species. What would happen if the levels of contamination at C. Palito decrease? Is this introduced species going to be able to extend its range? Or maybe, would *S. lomentaria* Pacific recolonize de area? All relevant questions, for which, more information is needed.

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FIGURE INDEX

CHAPTER 1

at the nodes indicate boostrap values based on 1000 replicates.

CHAPTER 2

TABLE INDEX

CHAPTER 1

CHAPTER 2